DETECTING METHOD AND DETECTION SUBSTRATE

FOR USE THEREIN

BACKGROUND OF THE INVENTION

5   Field of the Invention

The present invention is directed to examining multiple specimens at a time for multiple items, and provides a method in which matrix substrates with biological samples having different properties and

10   origins bound thereto are prepared, and on each matrix region, oligonucleotides having different sequences, proteins or drugs are spotted in an array, whereby multiple specimens are examined at a time for multiple items.

15   The present invention also relates to a method in which by using an oligonucleotide having a known base sequence as a detection probe to detect whether a complex is formed by intermolecular bond with this oligonucleotide, detection is made whether or not

20   components having a capability of bonding to the above described detection probe are contained, and a detection substrate having the oligonucleotide as a detection probe fixed on its surface, which is used exclusively for this detecting method.

25   Related Background Art

In identification of partial sequences included in the base sequence of a nucleic acid molecule, detection

of a target nucleic acid contained in a sample

originated from an organism or identification of genus

or species for various bacteria based on the

characteristics of the gene DNA of the bacteria, a

5 procedure may be used in which two or more probe DNAs

having known base sequences are used to detect whether

or not the nucleic acid molecule is a nucleic acid

molecule specifically binding to each probe DNA, namely

making hybridization with each probe DNA. As an

10 effective approach to performing speedily and

accurately examination of the two or more probe DNAs by

the hybridization method, a procedure is proposed in

which a probe array of two or more probe DNAs arranged

regularly on a solid phase is used to detect at a time

15 whether or not the nucleic acid molecule is a nucleic

acid molecule specifically binding to each probe DNA.

Among common methods for producing such probe

arrays, as described in European Patent No. 373203 (EP

0373203 B1) for example, methods are known in which

20 predetermined nucleic acid probes are synthesized in an

array form on a solid phase, and methods in which a

plurality of nucleic acid probes synthesized in advance

is supplied in an array form on the solid phase.

Prior technical documents disclosing the former

25 methods include, for example, U.S. Patent No.

5,405,783. Also, as one example of the latter methods,

a method in which cDNAs are arranged in an array form

on a solid phase using micropipetting is disclosed in, for example, U.S. Patent No. 5,601,980 and "Science", Vol. 270, pp. 467, (1995).

The probe array that is prepared with these

5   methods may be an array such that nucleic acid probes are arranged on a solid phase at a high density of 10000 or more probes per square inch. Hybridization reaction with multiple probes are carried out at a time by dipping this high-density probe array into a

10   specimen solution, and in so doing, the base sequence of genes is analyzed based on the base sequence of nucleic acids making hybridization. This method has an advantage that probes are arranged in a high density on a substrate of small area, thereby making it possible

15   to conduct multiple-item examination at a time with a small amount of samples to reduce burden associated with sampling from the subject.

As a method of preparing the high-density probe array for the above described application on the

20   substrate by the DNA synthesis process, a method in which a photolithography technology is applied is disclosed in the aforesaid U.S. Patent No. 5,405,783, but highly advanced equipment is required for implementing this method, and the method is not easy

25   enough for anyone to use.

Also, in the case where the number of specimens is large but the number of required examination items is

not so large, the integration degree of DNA probes on the probe array corresponding to the number of examination items does not need to be very high. Rather, there may be cases where it is necessary to

5 prepare a large number of probe arrays with a small number of desired DNA probes fixed, using a simpler method.

Actually, in the field of clinical examination, there are not necessarily many cases where examinations

10 for more than 10000 items are required. For example, in the case of group health examination and the like, there may be cases where it is more important to examine a large number of specimens with a limited number of items. For examining a large number of

15 specimens in this way, a system is required such that presence of diseases can be speedily examined through comparison with standard samples with respect to each specimen.

In addition, the amount of DNA specimen is

20 generally small as compared to that of oligonucleotide capable of being synthesized and used in the probe. For using it in a normal form in which the probe array substrate is dipped into the specimen solution for hybridization reaction, the amount of specimen DNA

25 allowing the substrate to be dipped sufficiently is required. Therefore, the size of the DNA probe array substrate is limited depending on the amount of

specimen DNA, and thus the array needs to be highly

dense. Alternatively, as a result of diluting the

specimen solution to ensure its volume for the size of

the probe array substrate, the concentration of DNA in

5   the specimen solution is reduced, and a procedure is

adopted of prolonging reaction time to make

compensation for the reduced concentration.

Also, since the amount of sampled specimens is

limited inherently because the specimen is an extract

10   from tissues, and it is subjected to pre-processing for

making a specimen solution for use in hybridization

reaction, specifically extraction of nucleic acid,

single-strand formation thereof, and process for

labeling, the amount of finally obtained samples is

15   very small. In order to make compensation for that,

the sample is subjected to processing for amplification

of the amount of DNA such as amplification processing

by PCR reaction before it is used for examination and

studies. However, there exists a disadvantage that

20   because primers separately prepared are required for

carrying out a PCR reaction, such processing can be

applied only to specific genes of which primer sequence

is known. In addition, there exist sequences that can

easily be amplified and sequences that can hardly be

25   amplified in the process of PCR reaction, and thus the

efficiency of reaction (rate of amplification) is not

uniform. For example, in the case where the content of

a specific mRNA in the total amount of extracted mRNA

is examined to determine diseases or like based on the

content, standard samples providing criteria should be

always prepared to make correction on the above

5   described amplification rate.

Although the amount of the specimen solution

required for hybridization reaction decreases as the

size of the substrate is reduced, there is a limitation

on downsizing of the substrate in association with

10   handling. Specifically, it is possible in principle to

enhance array density and reduce the number of probes

to be placed on the array to downsize the substrate,

but if an extremely small substrate is used, a

dedicated handling apparatus is required in the process

15   of processing such as hybridization reaction and

detection thereafter, which cannot be practical.

Also, for examining cDNA for mRNA that is

transcribed with reflection of the process of

development of a certain organism, cDNA for mRNA that

20   is transcribed with reflection of each phase in the

process of culturing a certain cell, cDNA for mRNA that

is transcribed by interaction with drugs, and so on, a

DNA array with multiple types of test samples arranged

is used. Examples of arraying this test sample are

25   described, for example, in the above described

"Science", Vol. 270, pp 460, (1995). In this case,

test samples arrayed on the substrate are dipped using

as a probe solution the labeled DNA of known sequence that is derived from genes having a specific function, whereby hybridization reaction is carried out.

5   If a plurality of items is to be examined at a time using this methodology, DNA probes labeled with different types of fluorescent reagents (fluorochromes) should be prepared depending on the number of items. When detection is made, those different types of fluorescent reagents (fluorochromes) must be detected

10 as distinguished from one another, and therefore their wavelengths and the like should be different as a matter of course. Of course, detection filters corresponding to respective fluorescent reagents (fluorochromes) are also needed for a detector.

15 This need for simultaneous examination of multiple items for multiple specimens is not characteristic exclusively of hybridization reaction among genes (DNA).

For example, it is also important to examine

20 multiple items with a small amount of samples as to interaction between genes and other substances such as interaction between genes and proteins (DNA binding proteins) and screening of chemicals that are bound to genes. Detection of former DNA binding proteins is

25 used to elucidate the control mechanism of gene expression by proteins such as transcription accelerators, but in the present situation, methods in

which DNA fragments are bound to proteins, and
thereafter complexes are analyzed by gel
electrophoresis are adopted. In this method, the
number of specimens that can be analyzed at a time is
5   limited due to usage of gel electrophoresis, and
considerable time is required for analysis.

For the field of development of drugs, there may
be cases where examination of interaction between genes
and administered drugs constitutes an important item in
10   progress of research, but it takes relatively much time
and efforts to obtain chemically synthesized products
for use in drugs to be researched, and it can be
considered that reduction in the amount of drugs to be
used in screening results in significant improvement in
15   efficient of their research.

As introduced above, there are cases where when a
complex is formed using interaction between two
substances such as hybridization between DNAs,
formation of a complex of DNA and a protein, and
20   interaction of a drug compound with gene DNA, or the
presence or absence of interaction causing a complex to
be formed is examined, the amount of samples of one of
those two substances is limited, and the limited amount
of samples should be used to conduct a series of
25   desired examinations across multiple types as to the
presence or absence of formed complexes. That is,
development of an examination method in which

consumption of samples required for individual examinations can be reduced to carry out examination across multiple types more efficiently within a limited amount of samples is desired.

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SUMMARY OF THE INVENTION

An object of the first invention is to provide a method of examining multiple specimens at a time for multiple items, for example a method in which matrix
10 substrates with biological samples having different properties and origins bound thereto are prepared, and on each matrix region, oligonucleotides or proteins having different sequences and drugs are spotted in an array form, whereby multiple specimens are examined at
15 a time for multiple items.

Another object of the invention is to provide a method in which multiple specimens can also be examined at a time for multiple items in a similar way for interaction between chemicals, especially drugs, and
20 cDNA, binding of proteins to cDNA and the like.

An object of the second invention is to provide a new method in which oligonucleotide of which base sequence is known and which can be obtained relatively easily is used as a detection probe, and when for a
25 limited amount of sampled specimens, the presence or absence of a bonding capability to the above described oligonucleotide as a detection probe or the degree of

the bonding capability is examined by the presence or
absence of complexes formed between those two
substances, or efficiency thereof is evaluated,
consumption of specimens required for evaluation for
5   each type of oligonucleotide as a detection probe can
be reduced. In addition, the invention also has an
object to provide a detection substrate with the above
described oligonucleotide being fixed as a detection
probe in a predetermined region of its surface, which
10   is used exclusively for the method, and provide a
method of preparing the detection substrate.

The examination method of the first invention
capable of achieving the above described objects is a
method in which a reactivity between a first sample and
15   a plurality of second samples having different
properties from one another is examined at a time,

characterized in that in a defined region on a
substrate with the first sample bound on the entire
surface in advance, the second samples are placed
20   independently of one another as spots having a smaller
size than the above described defined region, and then
the reactivity between the above described first sample
and each of the second samples is tested.

The matrix of biological samples related to the
25   invention that is usefully used for the above
examination method is characterized in that two or more
types of biological samples of different origins exist

in respective matrix regions separated on the substrate.

According to the invention, a substrate with biological samples having different properties and origins (e.g. nucleic acids and proteins) bound in a matrix form in advance can be provided.

There is also provided a method in which DNA probes like oligonucleotides, cDNAs, proteins or chemicals are spotted in an array form on the above described substrate with biological samples having different properties and origins placed in a matrix form to carry out reaction, and the presence or absence of another sample bound to a certain biological sample, the degree of the bonding, and the presence or absence of interaction is examined for multiple items at a time and speedily.

In this method, the area occupied by one specimen is very small because two or more types of specimens are placed on one substrate. Therefore, there is an advantage that the amount of required cDNA may be very small as compared to the case where hybridization reaction is carried out using a conventional DNA array with an enormously large number of DNA probes bound in an array form in advance. Also, there is neither limitation on the size of the DNA array substrate nor inconvenience for handling.

Also, by providing a method in which examination

can be carried out even with a small amount of samples,
the method opens the door to areas in which examination
could not be carried out because conventionally, a
sufficient amount of samples cannot be obtained, for
example a new examination area in which mRNA obtained
from tissues is directly examined.

In addition, according to the invention, a method
in which chemicals, proteins and nucleic acids can be
examined at a time under the same reaction condition on
the same substrate.

A method of detecting object components in test
samples according to the second invention is a method
in which using as a detection probe oligonucleotide of
which base sequence is known, complexes formed between
the above described oligonucleotide and the object
components are detected to examine whether or not the
object components having a capability of binding to the
above described oligonucleotide are contained in the
liquid test samples, or evaluate the degree of binding
capability thereof,

characterized in that there is at least one type
of the above described oligonucleotide used as a
detection probe, of which base sequence is known,

there are at least two types of test samples to be
examined, and

a detection substrate with the above described one
or more types of oligonucleotide for detection probes

bound to predetermined sections respectively on a
predetermined solid substrate is used,

the above described method comprising steps of:

spotting a plurality of predetermined amounts of
sample solution for each spot so that a predetermined
array shape is formed in the spotted position, for each
of the above described two or more types of test
samples, in each section with the oligonucleotide for
detection probes bound in advance;

detecting the presence or absence of complexes
formed between the above described oligonucleotide and
the object component, for the above described plurality
of spots for each test sample, respectively; and

determining whether or not the object component
having a capability of binding to the above described
oligonucleotide is contained, or the degree of the
capability of binding, based on the result of the above
described detection.

Also, the present invention provides a detection
substrate that is exclusively used when the above
described method of the invention is carried out. That
is, the detection substrate of the present invention is
a detection substrate with two or more oligonucleotides
having known base sequences different from one another
fixed on a solid substrate, characterized in that:

the above described plurality of oligonucleotides
are bound and fixed in predetermined sections,

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows one example of an arrangement aspect of defined regions on a substrate in the present invention;

5   FIGS. 2A and 2B show one example of matrices in the present invention, wherein FIG. 2A is a plan view, and FIG. 2B is a 2B-2B sectional view thereof;

FIG. 3 is a schematic explanatory view of a specimen solution discharging method by bubble jet

10   process that is an embodiment of the present invention;

FIG. 4 is a sectional view of a bubble jet head 105 taken in the 4-4 line in FIG. 3;

FIG. 5 shows a layout of 64 discharged DNA probes on each black matrix;

15   FIG. 6 shows one example of detection substrates of the present invention, illustrating schematically a situation in which sections in which oligonucleotides being detection probes are fixed are arranged in a matrix form, and a plurality of cDNAs are spotted in a

20   two-dimensional array form onto each section as detection samples;

FIG. 7 illustrates schematically arrangements of respective probes in the detection substrate with 64 DNA probes bound to sections arranged in the form of a

25   8 × 8 matrix, respectively;

FIG. 8 shows schematically a pattern of a spot array of total 64 × 64 in which 64 test samples are

respectively, so that one type of oligonucleotide exists in each section, and

a plurality of the above described sections in which oligonucleotides are fixed is placed in a matrix

5 form on the surface of the above described solid substrate.

The method of preparing the detection substrate of the present invention is a method suitable for preparation of the above described detection substrate

10 of the invention, and specifically is a method of preparing a detection substrate with two or more oligonucleotides having known base sequences different from one another fixed on a solid substrate, characterized in that:

15 for the above described solid substrate, a substrate with a plurality of sections separated in a matrix form in advance formed on the surface thereof is used,

the above described a plurality of

20 oligonucleotides is supplied into predetermined sections in predetermined amounts using printing by ink jet process, respectively, so that one oligonucleotide is present in each section, and

the supplied oligonucleotides are fixed in the

25 predetermined sections.

spotted in the form of a two-dimensional 8 × 8 array on each section, for the detection substrate on which sections with probes fixed therein are arranged in the form of the 8 × 8 matrix;

5   FIG. 9 shows schematically a result of spotting 64 test samples in the form of the two-dimensional 8 × 8 array on each section for 64 probes fixed in sections arranged in the form of the 8 × 8 matrix to carry out hybridization reaction; and

10   FIG. 10 shows an example of the structure of sections delimited by hydrophobic frame-structured walls provided on the detection substrate of the present invention, and arranged in the form of the 8 × 8 matrix.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

        One embodiment of the present invention will be described below referring to FIG. 1. FIG. 1 shows a substrate surface with 64 defined regions formed
20   thereon, wherein each region (matrix) measures 1 mm by 1 mm, and a space x between regions can be selected freely. For methods of preparing biological sample binding matrix substrates, for example, a method can be used in which the solution of a first sample (e.g.
25   biological sample) is printed on the entire surface of defined regions on the substrate as a "solid print pattern" by coating and ink jet processes, or is

supplied by methods such as chemical synthesis on the

substrate, and is bound in a matrix form on the

substrate through adsorption to the substrate or

chemical reaction between functional groups existing in

5   the biological sample and functional groups existing on

the substrate.   Furthermore, the situation in which the

first sample is bound on the entire surface of defined

regions means a situation in which the first sample is

bound across the entire surface such that when a second

10   sample and samples thereafter are supplied in these

defined regions, these reactions occur without being

limited to the positions in the above described regions

in which the samples are supplied.   For example, the

first sample may be fixed in layered form on the entire

15   surface, or the masses of molecules constituting the

first sample may be dispersed on the entire surface in

high density with micro-spaces being kept among them.

The defined regions on this substrate may

previously be provided on the substrate as a well

20   constituted by sections separated in pattern form by

walls of hydrophobic compounds.

Also, when using a substrate with nucleoside acid

(cDNA) being a biological sample fixed thereon as the

first sample, two or more probe DNAs possibly included

25   in cDNA are contacted with cDNA on the substrate as the

second sample and samples thereafter, and products of

reaction with the above described probes are detected

on the above described solid phase to detect the
presence or absence of probe DNA sequences in the above
described cDNA, two or more probes are supplied in an
array form as mutually independent spots in each matrix

5   with various kinds of cDNA bound in the defined
regions, thereby making it possible to perform
simultaneous detection with two or more probes.

Also, on the nucleic acid (cDNA) matrix, two or
more types of chemicals or proteins that are possibly

10   bound to cDNA are contacted with the probe DNA on the
substrate as mutually independent spots, thereby making
it possible to perform multiple-item examination
composed of these reactions at a time. Multiple-item
screening of DNA binding proteins and DNA binding

15   chemicals can be performed at a time by detecting
presence of binding of chemicals or proteins to probes
on the solid phase.

The present invention is characterized by
supplying probe DNA, proteins and chemicals in a form

20   of droplets of small amounts on the matrix on which
biological samples such as cDNA are applied, wherein
different types of samples are arranged in an array
form, thereby making it possible to perform
simultaneous multiple-item processing.

25   Combinations of the first sample fixed in advance
on the substrate and the second sample and sample
thereafter that are reacted with the first sample may

include the following combinations.

Specific examples of the matrix or the like formed of defined regions on the substrate for use in the present invention will be described below.

5   (Shapes of matrices with biological samples bound thereto)

The shapes of matrix patterns are not particularly limited, and may include any shapes, but shapes such as linear, squares and rectangular are preferable in that

10   they can be treated irrespective of how specimens are supplied, in consideration of convenience at the time of supplying specimens on the created substrate. Of course, forms such as circles and ellipses will cause no problems.

15   Materials that are fixed to the substrate as a first sample may include unknown base sequences derived from organisms, cDNA libraries, mRNA libraries, sets of two or more DNA and RNA, known DNA and RNA synthesized or derived from organisms or sets thereof, chips of

20   cloned oncogenes, protein fractions including at least one type of protein derived from organisms, proteins of single type, mixtures of known proteins of different types, and chemicals.

(Density of matrices with biological samples

25   bound)

The density of matrices is not particularly limited, but for a preferred form, the density of 400

per centimeter square is preferable. For density of
$400/cm^2$, the size of one matrix is a 500 μm square in
the case of square form. If samples to be arranged as
spots on the array are arranged as spots with diameters

5   of 100 μm, 25 spots are arranged in total with 5 spots
high by 5 spots wide. Also, if the diameter of sample
solution is 20 μm, the number of spots that can be
arranged in a row is 25, and 625 spots can be arranged
in total.

10   (Preparation of a substrate with biological
samples bound thereto)
       Samples originated from organisms (biological
samples) include nucleic acids and proteins. Nucleic
acids include, for example, mRNA and cDNA, and methods

15   for binding them on the substrate include a method in
which nucleic acid extracted and purified in advance is
applied to the substrate to fix the nucleic acid by
adsorption and electrostatic bond, and a method in
which the nucleic acid is fixed by providing covalent

20   bond thorough chemical reaction with functional groups
on the substrate using amino groups the nucleic acid
has.
       The method using negative electric charges of DNA
is a method in which nucleic acid is electrostatically

25   bound to a solid carrier subjected to surface treatment
with poly positive ions such as polylysine,
polyethyleneimine and polyalkylamine, and then blocking

of excessive positive ions is carried out, which is generally used.

(Types of functional groups of solid phases and nucleic acids)

5   Combinations of functional groups that are used for fixation include, for example, a combination of epoxy groups (on solid phase) and amino groups (amino groups in nucleic acid probe terminals or base groups). Methods for introducing epoxy groups to the solid

10   surface include, for example, a method in which polyglycidyl methacrylate having epoxy groups is applied to the solid surface composed of resin, and a method in which a silane coupling agent having epoxy groups is applied to the solid surface made of glass

15   and is reacted with glass.

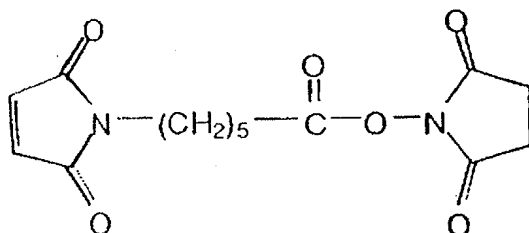(Binding of proteins to the solid phase)

Methods of binding proteins to the substrate include methods using adsorption as in the case of nucleic acid and methods using electrostatic binding.

20   Furthermore, methods of forming covalent bond include methods using SH groups of cysteine residues in addition to the above described methods using amino groups.

(Methods of fixation of proteins using thiol

25   groups)

Methods using cysteine residues for fixation of proteins include, for example, methods using

combinations of maleimide groups and thiol groups (-SH). That is, treatment is done so that the solid surface has maleimide groups, whereby thiol groups of cysteine residues supplied to the solid surface can be

5 reacted with maleimide groups of the solid surface to fix proteins.

For methods of introducing maleimide groups to the solid surface, a various kinds of methods may be used, and this can be achieved by, for example, reacting an

10 aminosilane coupling agent with a glass substrate, and then reacting its amino groups with a reagent containing N-(6-maleimidocaproyloxy) succinimide) expressed by the following structural formula (EMCS reagent: manufactured by Dojin Co., Ltd.).

15

For another example, a reagent containing

20 succinimidyl 4-(maleimidophenyl) butyrate can be used to react with amino groups preferably.

(DNA matrix structures composed of hydrophobic matrices)

For an additional form of fixation of biological

25 samples, a method can be used in which a well composed of, for example, hydrophilic and hydrophobic matrices is formed on the solid surface, a structure to prevent

coupling among spots is provided in advance, and the DNA prove is supplied in the well to carry out coupling reaction.

(Materials of matrices/wells)

5   When prove solution is put on the separated matrix to carry out coupling reaction, it is preferable that portions constituting the well is hydrophilic, and portions corresponding to the wall surface of the well and the partition between the well and a neighboring

10   well are composed of materials whose surfaces are less compatible with the prove solution. Due to such a treatment, the probe solution can be smoothly supplied to a desired well even if some positional deviation occurs when the prove solution is supplied to the well.

15   One example of matrices in this embodiment is shown in FIGS. 2A and 2B. FIG. 2A is a plan view, and FIG. 2B is a 2B-2B sectional view thereof. This matrix has a structure in which a matrix patter 125 having a frame structure with formed recesses 127 (wells) placed

20   in the form of a solid phase 103 is provided. The wells 127 separated from one another by the matrix 125 (height) are provided as through-holes (cut-off portions) in the matrix pattern, of which side is constituted by heights and of which bottom 129 has the

25   exposed surface of the solid phase 103. The portion of the exposed surface of the solid phase 103 forms a surface that can be coupled to the probe, and the probe

is fixed in a predetermined recess.

Materials forming the matrix pattern include, for example, metals (chrome, aluminum, gold, etc.) and resins. They include resins such as acryl,

5   polycarbonate, polystyrene, polyimide, acrylate monomers and urethane acrylate, and photosensitive resins such as photoresists having black dies and black pigments contained therein. For specific examples of photosensitive resins, UV resists, DEEP-UV resists,

10 ultraviolet cured resins and the like can be used. UV resists may include negative resists such as cyclized polyisoprene-aromatic pisazide resists, phenol resin-aromatic azide compound resists, and positive resists such as novolac resin-diazonaphthoquinone resists.

15 DEEP-UV resists may include, for example, radiation dispersion type polymer resists such as polymethyl methacrylate, polymethylene sulfone, polyhexafluorobutyl methacrylate, polymethyl isoprobenil ketone and bromo poly 1-

20 trimethylcylilpropine, and dissolution inhibiting resists such as cholate o-nitrobenzyl ester as positive type resists, and may include polovinylphenol-3-3'-diazidediphenylsulfone, and polymethacrylate glycidyl as negative type resists.

25 Ultraviolet cured resins may include polyester acrylate, epoxy acrylate and urethane diacrylate containing approximately 2 to 10% by weight of one or

more types of photopolymerization initiators, which are selected from benzophenone and substituted derivatives thereof, oxime compounds such as benzyl, and so on.

For curbing reflection by the material forming the matrix during detection, light-blocking materials can be effectively used for materials forming the matrix pattern. For this purpose, it is effective to add black pigments in the above described resin, and for black pigments, carbon black and black organic pigments can be used.

Here, if the matrix 125 is composed of resin, the surface of the matrix 125 is hydrophobic. This structure is preferred when aqueous solution is used as a solution containing probes to be supplied to the well. That is, even if the prove solution is supplied to the well, the prove solution is supplied to a desired well quite smoothly. Also, if different probes are supplied among adjacent wells at a time, intermingling (cross contamination) of different probe solutions supplied among these wells can be prevented.

The thickness of the matrix (height from the solid surface) is determined in the light of matrix pattern forming process and the volume of the well, but it is preferably in the range of 1 to 20 μm. Particularly, it can be considered as a thickness range allowing cross contamination to be prevented effectively when the probe solution is supplied to each well though an

ink jet process.

(Types of samples to be spotted)

Samples to be spotted as droplets onto the above described matrices of biological samples include probe nucleic acids, proteins and chemicals such as drugs.

For probe nucleic acids, in addition to deoxyribonucleic acid, any types of nucleic acids such as ribonucleic acid and peptide nucleic acid may be used as long as they have nucleic acid bases. The length of the oligonucleotide probe is not particularly limited, but it is preferably in the range of 10 mer to 50 mer for carrying out accurate hybridization reaction with cDNA.

For proteins, their own fluorescence can be used to detect DNA bonding proteins.

Some chemicals can also be detected with their own fluorescence.

(Method of preparing sample arrays)

Methods of spotting sample solution on defined positions in the size of several tens to several hundreds of microns include a pin system, an ink jet system and a capillary system.

The pin system refers to a method in which the sample is attached to the pin tip, for example, in such a manner that the pin tip is contacted with the surface of the solution including the sample, and then the tip is mechanically contacted with the solid phase, thereby

preparing a sample array. The capillary system using a capillary is such that the sample solution once sucked up to the capillary is mechanically contacted with the solid phase through the tip of the capillary as in the case of the pin system, thereby supplying the sample solution in an array form. For these spotting operations, various apparatuses commercially available from various companies may be used. These methods are considered as most preferable methods in the sense that any sample DNA can be supplied. However, as for quantification, the problem may be unsolved that viscosity varies depending on the length and concentration of DNA. For proteins, these methods are also preferred in the sense that they are deposited independently of the size and viscosity of molecules, but not suitable for quentitative analysis.

(Outline of sample array preparing methods through the ink jet process)

Samples capable of being discharged in an ink jet process include chemicals in addition to nucleic acids and proteins.

In the ink jet process, because shearing force is exerted, the length of dischargeable nucleic acids and the size of dischargeable proteins are often limited. However, it is superior in quantification to the pin system and capillary system, and is used more suitably than other systems with respect to discharge of

chemicals. Dischargeable nucleic acids are limited to those with relative length to bases of 5 kb or smaller, and dischargeable proteins are limited to those of 1000 K daltons or less. As for chemicals, any chemicals can

5   be discharged.

For liquids for discharge to be used for discharging and supplying samples with ink jets, any liquid can be used as long as it is capable of being discharged from ink jets, and the above described

10   liquid discharged from the head is shot in a predetermined position, and in the state of being mixed with nucleic acid probes and during discharge, the above described nucleic acid probes are not damaged.

And, in terms of dischargeability from the ink

15   jet, particularly from the bubble jet head, for the properties of the above described liquid, it is preferable that its viscosity is in the range of 1 to 15 cps and its surface tension is 30 dyn/cm or larger. Also, if the viscosity is in the range of 1 to 5 cps

20   and the surface tension is in the range of 30 to 50 dyn/cm, the position in which the liquid is spotted on the solid phase is extremely accurate, allowing the method to be used particularly suitably.

Therefore, if the stability of nucleic acid during

25   discharge or the like is taken into consideration, a nucleic acid probe of, for example, 2 to 5000 mer, particularly 2 to 1000 mer is preferably contained in

the solution in concentrations of 0.05 to 500 µM,

particularly 2 to 50 µM.

FIG. 3 is a schematic explanatory view of a

specimen solution discharging method through the bubble

5   jet process that is one embodiment of the present

invention. In FIG. 3, reference numeral 101 denotes a

liquid supplying system (nozzle) retaining a solution

including a specimen as discharge liquid in such a

manner that the solution is capable of being

10   discharged, reference numeral 103 denotes a solid phase

having a nucleic probe bound thereto with which the

above described specimen is reacted, and reference

numeral 105 denotes a bubble jet head having a function

of giving heat energy to the above described liquid to

15   discharge it, which is a type of ink jet head.

Reference numeral 104 denotes a liquid including the

specimen discharged from the bubble jet head. FIG. 4

is a 4-4 line sectional view of the bubble jet head 105

in FIG. 3, and in FIG. 4, reference numeral 105 denotes

20   the bubble jet head, and reference numeral 107 denotes

a liquid including a specimen solution to be

discharged, and reference numeral 117 denotes a

substrate portion having a heat generation portion to

give discharge energy to the above described liquid.

25   The substrate portion 117 includes a protective layer

109 formed by silicon oxide and the like, electrodes

111-1 and 111-2 formed by aluminum and the like, an

exothermic resistor layer 113 formed by nichrome and
the like, a heat storage layer 115, and a support 116
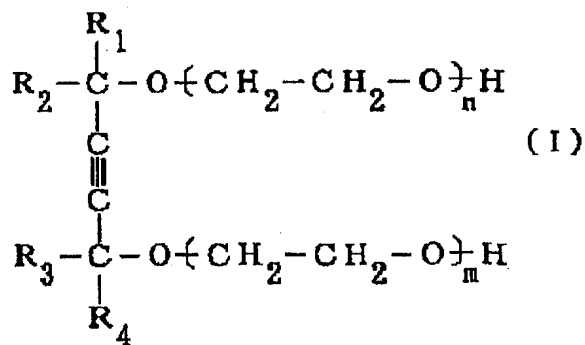formed by aluminum having good heat-release property.

The liquid 107 including the specimen comes to a

5   discharge orifice (discharge outlet) 119, and forms a
meniscus 121 with a predetermined pressure. Here, when
electric signals are applied to the electrodes 111-1
and 111-2, a region (foaming region) denoted by
reference numeral 123 abruptly releases heat, and the

10   liquid 117 contacted therewith is discharged and flies
toward the solid surface 103. The amount of liquid
that can be discharged using a bubble jet head having
such a structure varies depending on the size of its
nozzle, but can be controlled approximately to 4 to 50

15   picoliters, which is extremely useful as means for
placing specimen probes in high density.

And, in terms of dischargeability from the ink
jet, particularly from the bubble jet head, for the
properties of the above described liquid, it is

20   preferable that its viscosity is in the range of 1 to
15 cps and its surface tension is 30 dyn/cm or larger.
Also, if the viscosity is in the range of 1 to 5 cps
and the surface tension is in the range of 30 to 50
dyn/cm, the liquid is spotted in an exceedingly

25   accurate position on the solid phase, allowing the
method to be used particularly suitably.

Therefore, if the stability of nucleic acid during

discharge or the like is taken into consideration, a

nucleic acid of, for example, 2 to 5000 mer,

particularly 2 to 1000 mer is preferably contained in

the solution in concentrations of 0.05 to 500 µM,

5   particularly 2 to 50 µM.

For the composition of discharged liquid, the

composition of liquid is not particularly limited, as

long as the liquid has no substantial influence on the

nucleic acid probe when it is mixed with the nucleic

10   acid probe and when it is discharged from the ink jet,

and it can be normally discharged to the solid phase

using the ink jet, but preferable are liquids including

glycerin, urea, thiodiglycol or ethylene glycol,

isopropyl alcohol, and acetyl alcoholene expressed by

15   the following formula.

$$R_2-\underset{\underset{\underset{R_3-\underset{R_4}{\overset{|}{C}}-O(CH_2-CH_2-O)_m H}{\overset{\|}{C}}}{\overset{|}{C}}}{\overset{\overset{R_1}{|}}{C}}-O(CH_2-CH_2-O)_n H \qquad (I)$$

20

(In the above formula (I), R1, R2, R3 and R4

represent alkyl groups, specifically linear or branched

25   alkyl groups having 1 to 4 carbon atoms, m and n

represent integer numbers, respectively, wherein m and

n equal 0, or $1 \leq m + n \leq 30$ holds, and if m + n = 1 holds,

m or n equals 0).

Further specifically, a liquid containing 5 to 10% by weight (wt%) of urea, 5 to 10 wt% of glycerin, 5 to 10 wt% of thiodiglycol, and 0.02 to 5 wt%, more preferably 0.5 to 1 wt% of acetylene alcohol presented by the above formula (I) is suitably used.

The detecting method of the present invention is a method of detecting a complex formed between oligonucleotide for detection probes and an object component, which is used for the purpose of making evaluation/examination as to whether or not a component having capability of binding to oligonucleotide for use as a detection probe whose base sequence is known, and forming therewith a complex exists in a liquid test sample, and as to the degree of binding capability thereof if such a component exists in the sample. For detecting this complex, oligonucleotide for detection probes is fixed in advance on the solid surface substrate, whereby this fixed oligonucleotide is bound to the object component contained in the test sample, and the formed complex is separated while it is fixed on the solid substrate, and on the basis of a methodology for detecting complexes using proper detecting means, the amount of test samples required at this time is reduced to a very low level, and also, the detection accuracy and sensitiveness are kept at a sufficiently high level.

That is, in this methodology providing a base for the present invention, since surface density of the oligonucleotide for detection probes that is fixed on the solid surface substrate can be kept at a

5   predetermined value, the amount of the formed complex is proportional to the binding capability of the object component, and is also proportional to the concentration of the object component contained in the test sample that is contacted with the solid surface

10   substrate and is made to act on the oligonucleotide. Taking advantage of this characteristic, the test sample is contacted only with the surface with the oligonucleotide for detection probes actually fixed thereon, and the contact area is limited to a certain

15   level, whereby the amount of used test sample is also limited to a certain level. Specifically, by adopting means for spotting a predetermined minimal amount of liquid in the form of droplets, the contact area and the amount of liquid put thereon is controlled with

20   good reproducibility. The amount of the complex that would be automatically fixed on the solid surface substrate with formation is detected for this limited contact area, thereby achieving detection accuracy and sensitiveness essentially as high as those in the case

25   of dipping in the liquid test sample the whole of the solid substrate with oligonucleotide for detection probes fixed thereon.

The complex is detected by the label bound on the surface of the substrate. When a complex of the oligonucleotide and the labeled test sample is formed and the individual spots are sufficiently spaced from

5   each other, detection can be carried out independently for each spot. Therefore, if given or larger spaces are provided between adjacent spots, even though there are spots for different test samples nearby, only spots for desired test samples can be selected to continue

10   detection work without being influenced by those spots. In the detection method of the present invention, in order to satisfy reliably this requirement that given or larger spaces be provided between adjacent spots, a defined array is formed in the spot position as a

15   result of providing predetermined spaces as spaces between spots, and a predetermined amount of sample solution is spotted for each spot to make the spot area (contact area) constant, or make the spot diameter constant to ensure reproducibility because the shape of

20   the spot (contact surface) is generally a circle. As a matter of course, for precluding influence of adjacent spots, a space between spots is selected such that optical signals (fluorescent) and the like derived from the adjacent spots are not mixed in the detection

25   system, in the light of the measured area (diameter in the measurement range) of the detection system selected as appropriate in accordance with the spot diameter.

Also, as a matter of course, the detecting method of the present invention really shows its advantages in the case where there exist two or more types of test samples, and they are detected simultaneously.

5   On the other hand, on the surface to which a plurality of spots of such array forms is provided, one type of oligonucleotide for detection probes should be fixed in uniform surface density. Also, for the section in which the oligonucleotide for detection

10   probes is fixed, its area and shape are selected as appropriate in accordance with the above described array space and the total number of spots to be included in a series of arrays. It is also possible to provide sections having different oligonucleotides

15   fixed therein in different regions on the detection substrate to be used, and place a plurality of sections with two or more oligonucleotides fixed therein, respectively. That is, it can be said that the detecting method of the present invention becomes a

20   more suitable method if used when two or more types of nucleotides are used as detection probes to carry out a series of evaluations simultaneously for a plurality of test samples, with respect to two or more types of object components corresponding to respective

25   oligonucleotides.

Generally, in such an evaluation, it is often the case not that the oligonucleotides for detection probes

are predetermined while only an approximate number of

test samples to be evaluated is determined. In such a

case, it is preferable that as a detection substrate

with oligonucleotides for detection probes fixed

5   thereon in advance, a detection substrate with two or

more types of detection probes put thereon

systematically, having on the substrate surface in a

matrix form sections in which respective

oligonucleotides are fixed. In this detection

10   substrate with fixed sections arranged thereon in a

matrix form, the unit of total number of spots that are

made in an array form in each section is fixed, but a

plurality of these units of number of spots can be used

to carry out evaluation depending on the number of test

15   samples to be actually evaluated, thus enhancing

convenience in practice. Furthermore, for the each

section arranged in a matrix form, a pattern formed by

hydrophobic compounds is preferably provided in its

substrateer to provide a form in which mutual regions

20   are separated from one another.

In the detecting method of the present invention,

nucleic acid molecules may be selected as object

components to apply the same to evaluation as to

whether or not they are engaged in double-strand

25   formation into hybrid substances through hybridization

reaction with oligonucleotide for detection probes. In

this case, the method is an effective method in which

evaluation is made at a time even for multiple test samples, as to whether or not nucleic acid molecules including base sequences complementary to known base sequences that oligonucleotide for detection probes has

5   are contained in the test sample. Alternatively, if two or more types of nucleotides for detection probes are provided, and one type of nucleic acid molecules are contained in each test sample, evaluation can be made for the nucleic acid molecule of which base

10   sequences are still unknown, as to whether or not the nucleic acid molecule includes base sequences complementary to known base sequences that each oligonucleotide has, which is effective, for example, for means for making search for a gene group having a

15   set of homologies.

The detection substrate of the present invention is a DNA probe substrate with oligonucleotides for use in probes respectively bound to sections arranged in a matrix form in advance, and particularly for the

20   substrate itself, the bottoms of sections separated by wells (walls) of frame structure matrix patterns formed in advance by hydrophobic compounds are formed as hydrophilic surfaces, thereby making the binding of oligonucleotide easier. Also, by providing this

25   hydrophobic wall, intermingling of DNA probes among adjacent sections can be curbed more reliably.

Also, using these DNA probe substrates, the test

sample is spotted in an array form on the matrix of oligonucleotide to carry out hybridization reaction, thereby providing means for checking quickly whether or not nucleic acid molecules having complementarity are

5   included in each test sample for a certain oligonucleotide probe.

In this method, since the number of test samples that are used in hybridization reaction is determined depending solely on the number of spots, the size of

10   the detection substrate is not limited, and by using a substrate of large area, the section in which each probe is fixed can be widened and necessity to enhance density can be eliminated. Thus, since the section in which each probe is fixed can be widened, a wide range

15   of methods can be used such as methods in which a liquid containing probes is applied to, or printed as a "solid printed pattern" through ink jet process on defined regions on the substrate, or methods in which chemical synthesis is carried on the substrate, for

20   means for binding of the probe/oligonucleotide on the substrate.

Also, considering that the probe/oligonucleotide is less expensive and easier to obtain than the test sample, no significant problem arises even if the area

25   of the region to which oligonucleotide is bound is more or less increased, and in this case, with respect to a various kinds of test samples to be spotted, it is not

necessary to always spot them in high density.
Furthermore, when the test sample is spotted in small
amounts, the concentration of the object component that
is contained in the test sample is increased, whereby

5   hybridization reaction can be accelerated, thus making
it possible to perform high sensitive detection for a
short time. In addition, application of the detecting
method of the present invention will open the door to
fields that could not be considered previously because

10   a sufficient amount of samples could not be obtained,
for example a new field in which mRNA obtained from
tissues is directly examined.

Furthermore, information of reactivity in
association with obtained hybridization reaction is

15   analyzed/evaluated in terms of existence/not existence
of complementarity to various kinds of
oligonucleotides/probes, with respect to nucleic acid
molecules contained in a specific test sample, thereby
making it possible to carry out detection having also

20   functions similar to those of conventional DNA arrays
(hybridization reaction with multiple probes for one
specimen).

Furthermore, the detecting method of the present
invention provides means for evaluating as object

25   components the interaction between chemicals, in
particular drugs and oligonucleotides, the bonding of
proteins to oligonucleotides and the like, and

therefore, it can also be used as means making it possible to examine object components included in the test sample for multiple items, with respect to a large number of test samples. In addition, it provides means

5   making it possible to carry out examination on the same substrate at a time and under same conditions even for object components of different properties chemicals, proteins and nucleic acids.

The detecting method of the present invention and

10   the detection substrate for use exclusively therein will be described further in detail below.

In FIG. 6 is shown an example of applying the detecting method of the present invention to an embodiment in which using cDNA as an object component,

15   a hybrid substance is formed through hybridization reaction with oligonucleotide of known base sequence that is used for detection probes. In the detection substrate shown in FIG. 6, a plurality of rectangular sections separated systematically in a matrix form in

20   advance is provided on the solid surface substrate of rectangular form. The rectangular sections are each spatially isolated by matrix compartments that are surrounding walls. DNA probes that are used for hybridization/probes are each bound uniformly to the

25   bottom surface of the rectangular sections.

Also, attached is an enlarged view showing schematically a situation in which a plurality of test

samples including cDNA as an object component, for example two or more types of cDNA solutions prepared based on m-RNAs collected respectively are spotted in the form of the two-dimensional array like a square

5   matrix, in a section with the DNA prove fixed therein. The detection substrate, the detection probe, components to be detected and the like that are used in the detecting method of the present invention will be described further in detail.

10   (Oligonucleotides that are used for detection probes)

In the detecting method of the present invention, deoxyribonucleic acid can be used for oligonucleotide that is used for detection probes. In addition

15   thereto, ribonucleic acid, peptide nucleic acid and the like can be used. Types thereof are not limited as long as they have desired base sequences, and are capable of being bound to other molecules in those portions, and also as long as they can be fixed on a

20   solid substrate. Also, for portions excluding nucleic acid chains, those modified with non-nucleic acid atom groups and those having additional structures and so on can also be used as long as the above described requirements are satisfied.

25   Furthermore, for this oligonucleotide that is used for detection probes, a desired amount thereof should be artificially prepared or collected, and its base

sequence itself should be known. However, its nucleic

acid part should have at least two bases. Its base

length is not limited in principle, but if the length

exceeds that of 100 bases, difficulty becomes more

5   significant as its base length increases when fixation

on the solid substrate is to be carried out, and

therefore the base length is preferably restricted to

that of 100 bases or less.

For example, when this oligonucleotide is

10   subjected to hybridization reaction with, for example,

nucleic acid molecules with the length of more than 100

bases, the length of the oligonucleotide is preferably

at least 10 mer for obtaining sufficient bonding. On

the other hand, if the length exceeds 50 mer, it is

15   difficult to set conditions for controlling detection

of mismatching, thus making it difficult to select and

detect only those that are fully matched. Thus, in

order to detect mutations, the length is preferably 60

mer or smaller.

20   Furthermore, the range of 10 mer to 60 mer is a

preferable range even when oligonucleotide having

desired base sequences, for example DNA is prepared

through chemical synthesis.

(Shapes of sections with oligonucleotide fixed

25   therein, which is arranged in a matrix form)

The shape of a section itself in which

oligonucleotide for detection probes is bound and fixed

is not particularly limited. However, if considering

that a test samples is spotted in an array form on this

section, generally a simpler shape rather than a

complicated outside shape is preferably selected. In

5   addition, also when oligonucleotide is bound and fixed,

generally a simpler shape is preferably selected for

providing uniform surface density in such a section ,

in terms of working efficiency and convenience.

Specifically, rectangular forms, for example, line

10   forms, squares and rectangles are preferably adopted.

Of course, in principle, forms whose perimeters are

formed by curves such as circles and ellipses do not

cause any problems.

On the other hand, in the detection substrate of

15   the present invention, when two or more

oligonucleotides that are used for detection probes are

put on one substrate, sections in which they are fixed

are preferably arranged in a matrix form, in terms of

working efficiency and convenience. Also, preferably,

20   the form of each section is unified, and its area is

also unified.

(Density of sections arranged in a matrix form)

The density of sections arranged in a matrix form

is selected as appropriate depending on the number of

25   oligonucleotides that are put on the detection

substrate at a time, but the density of 400 per

centimeter square or less is preferable. If the

density is 400/cm^2, and the form of each section is a square, the size of each section is a 500 µm square. If test samples are closely arranged in an array form as spots with diameters of 100 µm, 25 spots are

5　　arranged in total with 5 spots high by 5 spots wide. Also, if the diameter of the spot is 20 µm, the number of spots that can be arranged in a row is 25, leading to 625 spots in total. Since the detecting method of the present invention has more significant advantages

10　　when there are a large number of test samples and they are examined at a time, the final object of the invention will be more satisfactorily achieved if the density of the section that is arranged is selected so that at least the upper limit of the number of test

15　　samples that can be spotted approximately equals the above described value.

　　　　For example, when the detecting method of the present invention is applied to test samples including cDNA, the number of test samples to be examined,

20　　specifically the total number of types of cDNA often is approximately as many as 3600. In this case, if the diameter of the spot is 100 µm, the size of one section approximately equals a 6 mm square when 60 spots are arranged in rows and columns, respectively. Also, even

25　　if the diameter of the spot is 20 µm, the size of one section should be a 1.2 mm square. In this way, in the detection substrate for use in the detecting method of

the present invention, there are not a few cases of application objects where the density of sections that are arranged in a matrix form is preferably selected as 400 per centimeter square or less.

5   Furthermore, in the detecting method of the present invention, the test sample is spotted as droplets, and in the case where the diameter of spot is 100 μm, for example, the amount of liquid required for the droplet of one spot is about 25 picoliters. Even

10   if the number of probes for use in examination is selected as 400 (for example, the number of sections of the matrix to be provided on the substrate is 400) for this spot size, the total amount of liquid required for the whole spots may be no more than 10 nanoliters for

15   each test sample, thus making it possible to carry out objective examination items with a minimal amount of liquid.

Also, in the conventional method in which the detection substrate is dipped in the solution of the

20   test samples, the amount of required liquid is dependent on the size of the substrate, and thus if the amount of the test sample is essentially very small, the size of the substrate should be reduced in accordance with the amount of liquid, and it is

25   essential to highly integrate probes that are fixed on the substrate. On the other hand, in the detecting method of the present invention, the size of the

substrate itself can be freely selected without

allowing for the liquid amount the test sample.   In

addition, when oligonucleotide that is used for

detection probes is fixed, the surface density should

5   be uniformed as a matter of course, but it is not

necessary to highly integrate a plurality of probes to

fix them, thus making the fixing operation easier.

(Fixation of oligonucleotide on the substrate)

As means for fixing oligonucleotide that is used

10   for detection probes on the surface of the substrate, a

method in which oligonucleotide separately prepared in

advance is supplied in predetermined sections by

coating or printing to bind the oligonucleotide, or a

method in which each oligonucleotide, specifically a

15   DNA probe or the like is synthesized in solid phase on

the substrate to prepare originally bound DNA can be

used.   Furthermore, even in the case where the

oligonucleotide is not DNA but, for example,

ribonucleic acid or peptide nucleic acid, synthesis on

20   the substrate can be carried out to bind the

oligonucleotide as described later.

On the other hand, when oligonucleotide,

specifically DNA or ribonucleic acid, peptide nucleic

acid or the like separately synthesized or collected in

25   advance is used for detection probes, a process of

fixing the oligonucleotide by covalent bond or of

fixing it electrostatic coupling on the surface of the

substrate can be used.

(Synthesis of oligonucleotide on the substrate)

Synthesis of DNA on the substrate includes synthesis on the silicon substrate using photolithography as a methodology disclosed in U.S. Patent No. 5445934. The U.S. Patent No. 5445934 shows a method in which high density DNA probe arrays are prepared by dividing the surface of the silicon substrate into very small areas, and synthesizing DNA for probes. On the other hand, in the detection substrate for use in the detecting method of the present invention, for example, the size of the section in which each probe is fixed may be a 0.5 mm square or larger, and thus it is not always necessary to enhance density. However, also in the detection substrate of the present invention, photodecomposable protective groups, protective groups that are decomposed by chemicals and the like are bound to nucleic acid in advance, and processes of masking, light exposure and reaction are repeated, whereby DNA chains can be synthesized on each section using the methodology described in the U.S. Patent Publication No. 5445934 in which four types of nucleic acid bases are bound for each base to stretch the DNA chain having desired base sequences.

(Fixation of oligonucleotide synthesized or collected in advance)

As means for carrying out fixation using electrostatic coupling, a method in which polylysine, polyethyleneimine and polyalkylamineaone on the solid surface substrate are subjected to blocking using the negative charge of DNA is generally used.

However, in the case of oligonucleotide with base length of 60 or less that is not sufficiently long, the electric charge of its phosphate groups is also weak, and thus binding onto the substrate by the above described method is not necessarily strong. For this oligonucleotide whose base length is not sufficiently long, if a method in which oligonucleotide with functional groups for covalent bond introduced in the terminal of nucleic acid is synthesized in advance, the substrate is subjected to surface processing suitable for functional groups, and the above described functional groups are used to accomplish covalent bond is used, stronger binding can be achieved, which is more preferable.
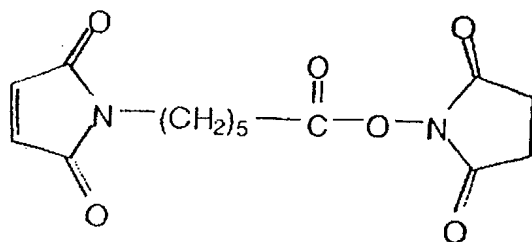
Also, in the case where the oligonucleotide is RNA, the above described method that is used for DNA may be applied. Alternatively, in the case where the oligonucleotide is peptide nucleic acid, its nucleic acid part may be used to apply the above described method that is used for DNA.

(Types of functional groups for use in fixation by covalent bond between the solid substrate and

oligonucleotide)

When oligonucleotide is fixed on the solid surface substrate though covalent bond, functional groups are generally introduced in oligonucleotide and the solid

5   surface substrate, respectively, in advance to carry out reaction therebetween. For this combination of functions, a preferable example is a combination such that maleimide groups are introduced in the surface of the substrate and thiol groups (-SH) are introduced in

10   oligonucleotide. Specifically, thiol groups (-SH) are bound to the terminal of oligonucleotide while the solid surface is subjected to processing of forming a coating having maleimide groups, and when oligonucleotide is supplied to the solid surface, the

15   thiol groups (-SH) are made to act on and react with the maleimide groups to perform fixation through formation of covalent bond.

For introducing maleimide groups in the solid surface, various kinds of methods may be used, and for

20   example, an aminosilane coupling agent is reacted with a glass substrate, and then a reagent (EMCS reagent: manufactured by Dojin Co., Ltd.) including N-(6-maleimidocaproyloxy) succinimide expressed by the following formula, whereby a coating layer having

25   maleimide groups can be formed.

$$O=\overset{\displaystyle O}{\underset{\displaystyle O}{\text{maleimide}}}N-(CH_2)_5-\overset{\displaystyle O}{\overset{\|}{C}}-O-N\overset{\displaystyle O}{\underset{\displaystyle O}{\text{succinimide}}}$$

5

For another example, a reagent containing

succinimidyl 4-(maleimidophenyl)butyrate can be used to

react with amino groups preferably.

Also, for example, oligonucleotide with thiol

10   groups introduced therein can be synthesized by using

5'-Thiol-Modifier C6 (manufactured by Glen Research

Co., Ltd.) as a five prime-end reagent when DNA is

synthesized using a DNA automatic synthesizing

apparatus. Furthermore, after synthesis, purification

15   processing by high speed liquid chromatography is

applied after normal deprotection reaction.

Combinations of functional groups capable of being

used for fixation by covalent bond include, for

example, a combination of epoxy groups (on the solid

20   surface) and amino groups (the terminal of

oligonucleotide) in addition to the above described

combination of thiol groups and maleimide groups.

Methods for introducing epoxy groups in the solid

surface include, for example, a method in which coating

25   is applied to the solid surface constituted by

polyglycidyl methacrylate having epoxy groups and a

method in which a silane coupling agent having epoxy

groups is applied to the solid surface made of glass
and is reacted with glass.

(Supply of oligonucleotide solution by the ink jet
process)

There is no particular limitation on means for
supplying predetermined sections on the surface of the
solid substrate with a solution containing
oligonucleotide to be fixed thereon, as long as a
uniform amount of liquid is supplied for each unit
area. In the case where printing by the ink jet
process and the like is used, a "solid print pattern"
is prepared, and then using an ink jet type printer
head that is used for ink jet printers, the cartridge
for the ink is filled with oligonucleotide solution
instead of the ink, and printing for a defined area is
carried out. If the amount of liquid to be supplied is
small, items of large volume like an ink cartridge are
not used, and instead a structure in which a sample
supplying portion such as a tube is connected to a head
to supply the oligonucleotide solution to the head may
be used.

For the oligonucleotide solution for discharge,
which is used in this method, a solution that is
capable of being discharged in the form of ink jets,
and has viscosity suitable for a minimal amount of
droplets discharged from the head to be shot onto a
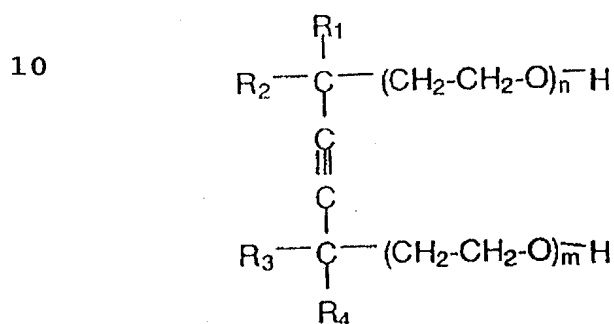desired position is used. In addition, a solvent to be

used is selected from solvents that satisfy the above
described requirements and give no damages to desired
oligonucleotide in the state of being mixed with the
desired oligonucleotide and during discharge.

5   Specifically, in terms of dischageability from the
ink jet head, particularly from the bubble jet head, it
is preferable that for example the viscosity is in the
range of 1 to 15 cps, and the surface tension is 30
dyn/cm or larger as the properties of the solution. In
10   particular, when the viscosity being in the range of 1
to 5 cps and the surface tension being in the range of
30 to 50 dyn/cm are selected, the position in which the
solution is shot onto the substrate is extremely
accurate, and a supplying method using the bubble jet
15   head is particularly suitably used.

In addition, if stability of oligonucleotide
during discharge and the like are considered, the
supplying means of the ink jet system is further
preferred when for example, a solution containing
20   oligonucleotide of 2 to 100 mer, particularly of 2 to
60 mer in concentrations ranging from 0.05 to 500 µM,
preferably from 2 to 50 µM is used.

In applying a discharging method of the ink jet
system, the liquid composition of the oligonucleotide
25   solution is not particularly limited, as long as the
solution gives practically no damages to desired
oligonucleotide in the state of being mixed with the

desired oligonucleotide and during discharge as a
matter of course as described above, and it can be
discharged to the surface of the solid substrate using
the ink jet. Furthermore, preferable is a solution

5   containing, for example, glycerin, urea, thiodiglycol
or ethylene glycol, isopropyl alcohol, or acetylene
alcohol expressed by the following formula in addition
to desired oligonucleotide.

10

$$R_2-\underset{\underset{\underset{\underset{\underset{R_4}{|}}{R_3-C-(CH_2-CH_2-O)_{\overline{m}}H}}{|}}{\underset{\|}{C}}}{\overset{\overset{R_1}{|}}{C}}-(CH_2-CH_2-O)_{\overline{n}}H$$

15

(In the above formula, R1, R2, R3 and R4 represent
alkyl groups, for example linear or branched alkyl
groups having 1 to 4 carbon atoms, respectively, and m
and n represent 0 or positive integer numbers,

20   respectively, and satisfy $1 \leq m + n \leq 30$). In addition,
specifically the liquid composition including 5 to 10
wt% of urea, 5 to 10 wt% of glycerin, 5 to 10 wt% of
thioglycol, and 0.02 to 5 wt%, more preferably 0.5 to 1
wt% of acetylene alcohol expressed by the formula (I)

25   allows the discharging method of the ink jet system to
be used suitably.

(Structure of matrices composed of hydrophobic

walls and hydrophilic wells)

Also, for sections of matrix form that are provided on the solid surface, for example, sections of matrix form constituted by hydrophobic walls (barriers)

5   surrounding hydrophilic wells (recesses) may be formed to prevent coupling between adjacent sections. A structure may also be used in which the solution of oligonucleotide is supplied to the hydrophilic wells (recesses) surrounded by the hydrophobic walls

10   (barriers), and oligonucleotide is fixed only in the bottom of the hydrophilic wells (recesses).

(Materials of walls/wells)

When as sections arranged in a matrix form, the solution of oligonucleotide is supplied to the bottom

15   of the wells (recesses) separated by wall (barrier) patterns to carry out binding reaction, it is desirable that the bottom of the wells (recesses) is wetted densely with the solution, but the walls (barriers) have poor wettability with the solution. For example,

20   it is preferable that the solid material constituting the surface of the bottom of the wells (recesses) is much hydrophilic, and the surface of the walls (barriers) and the portion corresponding to partitions with neighboring sections are less hydrophilic. The

25   solution of oligonucleotide supplied in the bottom of the well (recess) is spread across the bottom, but is prevented from finding its way over the wall (barrier)

into adjacent sections. Also, even the droplet

erroneously supplied in the position related to the

wall (barrier) quickly moves into a desired well

(recess) having good wettability, and as a result a

5   predetermined amount of oligonucleotide solution can be

supplied in the well (recess) more reliably.

An example of sections arranged in a matrix form

that is provided on the detection substrate of the

present invention is shown in FIG. 10. The sections in

10   a square matrix form have a structure in which heights

(walls) having frame structures are provided on the

surface of the solid substrate, and arranged

rectangular recesses (wells) are separated.

Specifically, the recesses (wells) separated from one

15   another by the heights (walls) having frame structures

are formed by coating the entire surface of the solid

substrate with a material forming heights (walls), and

thereafter providing rectangular through-holes (cut-off

portions) to open recesses (wells). Thus, the bottom

20   of the recess (well) has an exposed surface of the

solid substrate. The exposed portion of the surface of

the solid substrate is subjected to processing for

providing a surface to which oligonucleotide can be

bound. As a result, oligonucleotide is fixed only in

25   the bottom of this recess (well).

Materials forming heights (walls) having frame

structures include, for example, metals (chrome,

aluminum, gold, etc.) and resins. Resins include

resins such as acryl, polycarbonate, polystyrene,

polyimide, acrylate monomers and urethane acrylate, and

photosensitive resins such as photoresists having black

5   dies and black pigments contained therein.

Furthermore, for specific examples of photosensitive

resins, UV resists, DEEP-UV resists, ultraviolet cured

resins and the like can be used. UV resists may

include negative resists such as cyclized polyisoprene-

10   aromatic pisazide resists, phenol resin-aromatic azide

compound resists, and positive resists such as novolac

resin-diazonaphthoquinone resists.

DEEP-UV resists may include, for example,

radiation dispersion type polymer resists such as

15   polymethyl methacrylate, polymethylene sulfone,

polyhexafluorobutyl methacrylate, polymethyl

isoprobenil ketone and bromo poly 1-

trimethylcylilpropine, and dissolution inhibiting

resists such as cholate o-nitrobenzyl ester as positive

20   type resists, and may include borovinylphenol-3-3'-

diazidediphenylsulfone, and polymethacrylate glycidyl

as negative type resists.

Ultraviolet cured resins may include polyester

acrylate, epoxy acrylate and urethane diacrylate

25   containing approximately 2 to 10% by weight of one or

more types of photopolymerization initiators, which are

selected from benzophenone and substituted derivatives

thereof, oxime compounds such as benzyl, and so on.

When detection is carried out using a fluorescent mark, a light-blocking material can be used effectively for curbing light reflex by the material forming this height (wall) having a frame structure. For providing a light-blocking property, it is effective to add black pigments in the above described resins, and in this case, black pigments that can be used may include carbon black and black organic pigments.

Furthermore, if the height (wall) having a frame structure is formed by the above described hydrophobic resin, the surface of the height (wall) is hydrophobic. The configuration in which heights (walls) having frame structures that are formed by hydrophobic materials are provided is more preferable in the case where an aqueous solution is used as a solution containing oligonucleotide to be supplied to the surface of the substrate of recesses (wells). Even if the aqueous solution is supplied in a position related to the surface of the height (wall), it is not persistently attached to the surface of the wall, but gradually moves to the bottom of the recess (well) located in a lower position. Also, solutions of different oligonucleotides are supplied to adjacent recesses (wells), but they are separated from each other by the hydrophobic height (wall), and therefore intermingling (cross contamination) between solutions due to

penetration of liquid is prevented.

Furthermore, for the thickness (height from the solid surface) of the height (wall) having a frame structure, the volume of the recess (well) is selected

5   in the light of the amount of the oligonucleotide solution that is supplied to the recess (well), and the thickness is determined as appropriate so that the volume is filled with the solution. Also, depending on methods of forming the height (wall), the thickness is

10   preferable selected such that it is in the range of 1 to 20 μm and satisfies the above described requirement. The thickness of the height (wall) selected in this way is in the range of thickness allowing cross contamination between adjacent wells to be prevented

15   effectively when the oligonucleotide solution is supplied to each well by the ink jet process.

(Types of specimens)

Object components contained in the test sample to which the detecting method of the invention can be

20   applied include mRNA, cDNA, proteins, cell extracts and chemicals such as drugs.

Furthermore, when cDNA is used as an object component, it is possible to use double-strand cDNA directly, but the single-strand cDNA marked in advance

25   is preferable in forming hybrid substances efficiently and performing detection thereof conveniently.

On the other hand, mRNA is of essentially single-

strand, and it is marked in some way to form marked
mRNA, thereby making it possible to form hybrid
substances efficiently and perform detection thereof.
Furthermore, the amount of mRNA in the test sample is

5   generally small, and it is an object component more
remarkably reflecting the advantage that the amount of
sample solution required for detection can be reduced
to a low level, which is characteristic of the
detecting method of the present invention. However,

10   since admixture of RAN decomposition enzymes tends to
occur during handling, a predetermined amount of
substance to curb decomposition of mRNA such as RNA
decomposition enzyme inhibitors such as diethyl
pyrocarbonate is desirable added in the test sample

15   solution. In addition to mRNA, similarly, the genome
of RNA viruses can be an object component. In
addition, tRNA, ribosomal RNA and the like can be
object components.

On the other hand, when the protein is used as an

20   object component, formed complexes can be detected
using the fluorescence emitted by the protein itself.

Also, some chemicals also emit their own
fluorescence, and enables formed complexes to be
detected using the fluorescence. Chemicals that do not

25   emit fluorescence may be marked by methods using
functional groups of compounds. Those to which the
detecting method of the invention can be applied may

include, for example, chemicals that can be bound to single-strain DNA. In addition, they may include, for example, chemicals that can be bound to single-strain RNA.

5   (Means for spotting test samples in an array form)

In the detecting method of the present invention, the test sample is spotted in an array form in a defined position on the detection substrate. For the purpose of reducing amount of required liquid to a

10   minimal level, the spot diameter is selected so that it is in the range of several tens to 100 μm, but with such a spot diameter, the liquid should be spotted in high uniformity of spotted amounts and high positional accuracy. As means for satisfying this requirement,

15   there are spotting apparatuses of pin systems, ink jet systems and capillary systems.

The pin system refers to a method in which the test sample is attached to the pin tip, and the end point thereof is mechanically contacted with the solid

20   surface, thereby taking out a fixed amount of the test sample. The capillary system using capillaries refers to a method in which the test sample solution is sucked up to the capillary on a temporary basis, and the tip of the capillary is mechanically contacted with the

25   solid surface as in the case of the pin system, thereby taking out a fixed amount of the test sample. A various kinds of spotting apparatuses adopting these

two systems are commercially available, and thus commercially available apparatuses may be used.

The spotting apparatuses of the pin system and capillary system enable any types of test samples to be spotted, and are considered as most preferable methods for unknown test samples. For example, however, the viscosity of the test sample solution is varied depending on the length and the concentration of DNA contained in the test sample, and therefore the amount of spotted liquid is varied. Thus, a problem arises in terms of quantification. Also with respect to proteins, the viscosity of the test sample solution is varied depending on the size of the molecules and the concentration, thus raising a problem in terms of quantification.

(Spots in an array form of test samples by the ink jet process)

Specimens that can be discharged by the ink jet process include chemicals in addition to nucleic acids and proteins.

In the ink jet process, because shearing force is exerted, the length of nucleic acids and the size of proteins that can be discharged are limited. However, it is superior in quantification to the pin system and capillary system, and is used more suitably than other systems, particularly with respect to discharge of chemicals. Preferably, dischargeable nucleic acids are

those with relative length to bases of 5 kb or smaller,
and dischargeable proteins are those of 1000 K daltons
or less. As for chemicals, their molecular weights are
generally small enough compared to nucleic acids and

5   proteins, and therefore any chemicals can be discharged
except for polymers having extremely large molecular
weights.

FIG. 3 illustrates schematically a method of
discharging specimen solution by the ink jet process,

10   particularly the bubble jet process, which is one means
that is used for spotting test sample solution in the
present invention. In FIG. 3, reference numeral 101
denotes a liquid supply system (nozzle) retaining a
solution including a specimen as discharge liquid in

15   such a manner that the solution is capable of being
discharged, reference numeral 103 denotes a solid phase
having a nucleic probe bound thereto with which the
specimen is reacted, and reference numeral 105 denotes
a bubble jet head having a function of giving heat

20   energy to the liquid to discharge it, which is a type
of ink jet head. Reference numeral 104 denotes a
liquid (droplet) including the specimen discharged from
the bubble jet head. FIG. 4 is a sectional view of the
bubble jet head 105 described in FIG. 3. In FIG. 4,

25   reference numeral 107 denotes a liquid including a
specimen solution to be discharged from the bubble jet
head 105, and reference numeral 118 denotes a substrate

portion having a heat generation portion to give

discharge energy to the above described liquid. The

substrate portion 118 includes a protective layer 109

formed by silicon oxide and the like, electrodes 111-1

5   and 111-2 formed by aluminum and the like, an

exothermic resistor layer 113 formed by nichrome and

the like, a heat storage layer 115, and a support 116

formed by aluminum having good heat-release property.

The liquid 107 including the specimen comes to a

10   discharge orifice (discharge outlet) 119, and forms a

meniscus 121 with a predetermined pressure. In this

situation, when electric signals are applied to the

electrodes 111-1 and 111-2, a region (foaming region)

denoted by reference numeral 123 abruptly releases

15   heat, and the liquid 117 contacted therewith is

discharged and flies toward the solid surface 103. The

amount of liquid that can be discharged using a bubble

jet head having such a structure varies depending on

the size of its nozzle, but can be controlled

20   approximately to 4 to 50 picoliters, which is extremely

useful as means for arranging probes in high density in

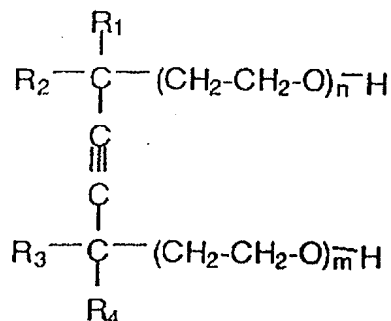a matrix form on the surface of the substrate.

And, in terms of dischargeability from the ink

jet, particularly from the bubble jet head, for the

25   properties of the above described liquid, it is

preferable that its viscosity is in the range of 1 to

15 cps and its surface tension is 30 dyn/cm or larger.

Also, if the viscosity is in the range of 1 to 5 cps and the surface tension is in the range of 30 to 50 dyn/cm, the position in which the droplet is spotted (spot position) on the solid phase is extremely

5   accurate, allowing the method to be used particularly suitably.

In addition, if the stability of nucleic acid during discharge or the like is taken into consideration, single-strain nucleic acid or double-

10   strain nucleic acid of, for example, 2 to 5000 mer, particularly 2 to 10000 mer is preferably contained in the solution. For example, c-DNA chips are preferably contained in the concentration of 0.05 to 500 µM, particularly 2 to 50 µM.

15   For the composition of discharged liquid, the composition of liquid is not particularly limited, as long as the liquid has no substantial influence on the nucleic acid probe when it is mixed with the nucleic acid probe and when it is discharged from the ink jet,

20   and it can be normally discharged to the solid phase using the ink jet, but preferable are liquids including glycerin, urea, thiodiglycol or ethylene glycol, isopropyl alcohol, and acetyl alcohol expressed by the following formula.

25

$$R_2 - \underset{\underset{\displaystyle \underset{\displaystyle R_3 - \underset{R_4}{\overset{|}{C}} - (CH_2\text{-}CH_2\text{-}O)_{\overline{m}}H}{\overset{|}{C}}}{\overset{\underset{\displaystyle \overset{|||}{C}}{\overset{|}{C}}}{\overset{R_1}{\overset{|}{C}}}} - (CH_2\text{-}CH_2\text{-}O)_{\overline{n}}H$$

(In the above formula, R1, R2, R3 and R4 represent alkyl groups, specifically linear or branched alkyl groups having 1 to 4 carbon atoms, m and n represent 0 or positive integer numbers, respectively, and satisfy $1 \leq$ + n$\leq$30 holds).

Further specifically, a liquid containing 5 to 10% by weight (wt%) of urea, 5 to 10 wt% of glycerin, 5 to 10 wt% of thiodiglycol, and 0.02 to 5 wt%, more preferably 0.5 to 1 wt% of acetylene alcohol is suitably used.

[Examples]

The present invention will be described in detail below using Examples. Furthermore, the Examples shown herein represent one example of most suitable embodiments of the present invention, but the invention should not be limited by these Examples.

Example 1

A glass substrate with black matrices for specimen matrices for analyzing sequences of p 53 genes on a specimen matrix substrate partitioned by patterns is

prepared.

    1.   Preparation of a black matrix introduction substrate coated with polylysine. A glass substrate (60 mm × 50 mm) made of synthetic quartz is subjected to supersonic cleaning using 2% sodium hydrate solution, and is then subjected to UV ozone processing to clean the surface. Then, a polylysine solution (manufactured by sigma Co., Ltd.) is applied to the entire surface with a spin coater. In addition, a DEEP-UV resist (negative type resist for black matrices) (BK-739P manufactured by Nippon Steel Chemical Co., Ltd.) is applied thereto with the spin coater so that the thickness after curing is 5 µm, and this substrate is heated for curing at 80°C for 5 minutes with a hotplate. Using a DEEP-UV aligner, a region of 1 cm × 1 cm is proximately exposed to light using a patterned mask so that the distance (X) between adjacent wells in FIG. 1 is 100 µm and the form of the well is a square of 1 mm × 1 mm, and then development is carried out with a developing solution of inorganic alkaline solution using a spin drier, and the developing solution is washed out completely with purified water.

    Then, the substrate is briefly dried using the spin drier, and is thereafter heated at 180°C for 30 minutes in a clean oven to have resist fully cured to obtain a substrate in which 400 wells are arranged as a

predetermined arrangement and adjacent wells are separated from each other by the black matrix. Furthermore, the volume of each well is calculated as 5 μl if the thickness of the liquid is 5 μm.

5   2. Fixation of specimen DNA

(1) Preparation of cDNA libraries

The p 53 gene is obtained by a PCR reaction from 64 types of cDNA libraries obtained form tumor tissues.

That is, RNA samples were obtained from each

10 tissue collected with biopsies using Catrimox-14 (Biotechnology Co., Ltd.). Based on this sample solution, First-Strand cDNA Synthesis Kit (manufactured by Life Sciences Co., Ltd) is used to obtain cDNA libraries.

15 (2) Amplification of p53 genes having T3 binding sites by a PCR method.

Based on the cDNA library, "Human p53 Amplimer set" manufactured by CLONTECH Co., Ltd. is used to carry out PCR reaction.

20 As a PCR reaction solution, "one shot LA PCR Mix" (Takara Shuzo Co., Ltd.) was used. The composition of the PCR reaction solution is as follows:

One shot LA PCR Mix   25 μl

5' primer (20 μM)   1

25 3' primer (20 μM)   1

cDNA library solution   1

DW   22/50 μl.

The PCR cycle is such that after thermal denaturation at 95°C for 5 minutes, cycles at 95°C for 30 seconds, at 55°C for 30 seconds and at 72°C for 60 seconds are conducted 29 times, and finally the solution is left for reaction at 72°C for 5 minutes and is then stored at 4°C.

After the reaction, gel electrophoresis is performed to confirm a product existing in the region of molecular weight of about 300 mer, and purification is carried out with MicroSpin Column S200 (Pharmacia) to obtain p 53 genes (p 53 DNA).

(3) Synthesis of single-strain p 53 DNA

Using as a matrix the DNA obtained in the above (2), a single-strain marked DNA is obtained by the PCR reaction using 5' primer (Takara Shuzo Co., Ltd.). The composition of the reaction solution comprises

One shot LA PCR Mix	25 µl
5' primer (20 µM)	1
P 53 DNA	1
DW	22/50 µl, and

the reaction cycle is such that cycles at 96°C for 30 seconds, at 60°C for 15 seconds and at 60°C for 4 minutes are repeated 24 times, and finally the solution is stored at 4°C. Thereafter, it is purified with MicroSpin Column S200.

(4) Fixation of p 53 cDNA

5 µl of the single-strain DNA obtained in the

above (3) is injected under a microscope into each well

of the polylysine-coated substrate with black matrices

prepared in the above (1), and is fixed through

electrostatic coupling.

5   3.   Analysis of variation of p 53 genes with

oligonucleotide probes

The 64 DNAs were selected, focusing attention on

the 248th and 249th amino acid sequences of the p 53

gene being a tumor inhibitor gene. That is, it is

10   known that a case of frequent variation in the base

sequence of CGGAGG is the case where the first C is

changed to T, the second A is changed to G, and the

third G of the sequence corresponding to the 249th

amino acid is changed to T. Thus, the 64 probes are

15   designed, focusing attention on the base sequence at

these three points.

That is, it is a structure in which the total

length of the probe is 18 mer, and six bases including

this variation are located at the center thereof, and

20   the bases are sandwiched between common sequences. A

common sequence corresponds to the range from the five

prime-end to the ATGAAC, and the subsequent portion

including variation corresponds to the NNGAGN and a

further subsequent common portion corresponds to the

25   CCCATC, resulting in a final sequence of

5'ATGAACNNGAGNCCCATC3'. Here, the portion expressed by

N corresponds to the A, G, C, and T that are four types

of nucleic acid bases. The probe DNA has a sequence complementary to the sequence to be detected (the above described sequence), and thus the sequence thereof is 5'MGGGNCTCNNGTTCAT3'. Rhodamine is coupled to the five prime-end of each probe sequence to mark the prove. Specific base sequences of these 64 types of marked DNA probes are shown in the following Table 1.

Table 1

SEQ ID NO.	Sequence	SEQ ID NO.	Sequence
1	GATGGGACTCAAGTTCAT	33	GATGGGCCTCAAGTTCAT
2	GATGGGACTCAGGTTCAT	34	GATGGGCCTCAGGTTCAT
3	GATGGGACTCACGTTCAT	35	GATGGGCCTCACGTTCAT
4	GATGGGACTCATGTTCAT	36	GATGGGCCTCATGTTCAT
5	GATGGGACTCGAGTTCAT	37	GATGGGCCTCGAGTTCAT
6	GATGGGACTCGGGTTCAT	38	GATGGGCCTCGGGTTCAT
7	GATGGGACTCGCGTTCAT	39	GATGGGCCTCGCGTTCAT
8	GATGGGACTCGTGTTCAT	40	GATGGGCCTCGTGTTCAT
9	GATGGGACTCCAGTTCAT	41	GATGGGCCTCCAGTTCAT
10	GATGGGACTCCGGTTCAT	42	GATGGGCCTCCGGTTCAT
11	GATGGGACTCCCGTTCAT	43	GATGGGCCTCCCGTTCAT
12	GATGGGACTCCTGTTCAT	44	GATGGGCCTCCTGTTCAT
13	GATGGGACTCTAGTTCAT	45	GATGGGCCTCTAGTTCAT
14	GATGGGACTCTGGTTCAT	46	GATGGGCCTCTGGTTCAT
15	GATGGGACTCTCGTTCAT	47	GATGGGCCTCTCGTTCAT
16	GATGGGACTCTTGTTCAT	48	GATGGGCCTCTTGTTCAT
17	GATGGGGCTCAAGTTCAT	49	GATGGGTCTCAAGTTCAT
18	GATGGGGCTCAGGTTCAT	50	GATGGGTTCTAGGTTCAT
19	GATGGGGCTCACGTTCAT	51	GATGGGTCTCACGTTCAT
20	GATGGGGCTCATGTTCAT	52	GATGGGTCTCATGTTCAT
21	GATGGGGCTCGAGTTCAT	53	GATGGGTCTCGAGTTCAT
22	GATGGGGCTCGGGTTCAT	54	GATGGGTCTCGGGTTCAT
23	GATGGGGCTCGCGTTCAT	55	GATGGGTCTCGCGTTCAT
24	GATGGGGCTCGTGTTCAT	56	GATGGGTCTCGTGTTCAT
25	GATGGGGCTCCAGTTCAT	57	GATGGGTCTCCAGTTCAT
26	GATGGGGCTCCGGTTCAT	58	GATGGGTCTCCGGTTCAT
27	GATGGGGCTCCCGTTCAT	59	GATGGGTCTCCCGTTCAT
28	GATGGGGCTCCTGTTCAT	60	GATGGGTCTCCTGTTCAT
29	GATGGGGCTCTAGTTCAT	61	GATGGGTCTCTAGTTCAT
30	GATGGGGCTCTGGTTCAT	62	GATGGGTCTCTGGTTCAT
31	GATGGGGCTCTCGTTCAT	63	GATGGGTCTCTCGTTCAT
32	GATGGGGCTCTTGTTCAT	64	GATGGGTCTCTTGTTCAT

Then, for each of the 64 types of marked prove

DNAs, a 8 μM solution containing glycerin, urea and

thiodiglycol in the final concentration of 7.5%, and

acetylenol EH in the final concentration of 1% is

5   prepared. A different probe solution is charged by 100

μl in each of the six nozzles of BJ Printer Head BC 62

(manufactured by Cannon Inc.). Arrangement is made so

that six DNAs can be discharged for each head, and two

heads are used to discharge 12 DNAs at a time, and the

10   heads are exchanged 6 times to discharge DNAs so that

each spot of 64 DNA is formed independently. In this

way, total 64 probes are discharged in the form of the

8 × 8 array in each well of black matrix coated with

polylysine.

15   FIG. 5 shows an arrangement on each black matrix

of 64 DNA probes that are discharged. In this case, 64

DNA probes are spotted in one matrix.

Thereafter, this substrate in which each probe is

spotted is left in a humidifier chamber set at 40° C to

20   carry out a hybridization reaction.

Thereafter, the substrate is cleaned with a 10 mM

phosphate buffer containing 100 mM NaCl to remove DNA

probes that have not been engaged in the formation of

the hybrid substance.

25   DNA arrays after the hybridization reaction are

observed using an inverted fluorescence microscope

equipped with a filter set suitable for rhodamine.

If the gene as a specimen has normal base sequences, spots of highest fluorescence intensity should be observed in the gene at the location of the relative 42th DNA prove. It can be considered that those are derived from the hybrid of the p 53 gene having normal sequences amplified with the probe DNA and PCR. In a varied gene, detectable spots are observed at the location other than the 42th location, and a varied sequence can be known from the DNA probe supplied to the location.

Example 2

(Evaluation of existence/not existence of carcinogenic genes using mRNA)

1. Extraction of mRNA

"QuickPrep Micro mRNA Purification Kit (manufactured by Amersham Pharmacia biotech co., Ltd.) is used to extract mRNA from tumor tissues collected with the biopsy. This mRNA is bound to a polylysine substrate with black matrices as in the case of Example 1.

2. Examination of existence/not existence of carcinogenic genes and the type thereof with various kinds of carcinogenic gene probe arrays.

Sets of cloned oncogenes (18 types, manufactured by Takara Shuzo Co., Ltd.) are purchased, and then "LabelITnon-RI Labeling Kits" are used to perform rhodamine marking.

18 types of marked oncogene probes are spotted as an arrangement of 4 × 5 on the above described substrate with mRNA bound thereto, using a microarray preparing apparatus (pin system) manufactured by

5   Cartesian Technologies Co., Ltd.

Further, a hybridization reaction is carried out as in the case of Example 1.

The type of oncogenes existing in the mRNA section extracted from each tissue can be known.

10   At this time, detection can be sufficiently performed with one type of marks irrespective of types of oncogenes.

The second invention will be describe more specifically with Examples below.

15   (Example 3)

An example of procedures for preparing an substrate with oligonucleotide bound thereto will be described below. In this embodiment, a detection substrate with oligonucleotide bound to a region of 2

20   mm square on a glass substrate was prepared in accordance with the procedure described below.

1. Cleaning of the substrate

A glass substrate of 1-inch square was placed in a rack, and was soaked in a detergent for ultrasonic

25   cleaning. Thereafter, it was subjected to ultrasonic cleaning in the above described detergent for 20 minutes, followed by removing the detergent by rinsing.

Furthermore, it was rinsed with distilled water,

followed by further performing ultrasonication for 20

minutes in a container containing distilled water.

Then, this glass substrate was soaked for 10

5   minutes in 1N sodium hydrate solution heated in

advance. After it was taken out from the solution, the

1N sodium hydrate solution adhered to the surface was

washed out with water, and thereafter cleaning with

distilled water was continued.

10   2.   Surface treatment

The above described cleaned glass substrate was

soaked in an aqueous solution of 1% silane coupling

agent (manufactured by Shin-Etsu Chemical Co., Ltd.,

Trade name: KBM 603) at room temperature for 20

15   minutes, followed by spraying nitrogen gas on the both

sides of the substrate to drive off water for drying.

The substrate was baked for one hour by using an oven

heated to 120°C to complete treatment of the surface of

the glass substrate with a silane coupling agent.

20   On the other hand, 2.7 mg of EMCS (N-(6-

Maleimidocaproyloxy) succinimide: manufactured by Dojin

Co., Ltd.) was weighed, and was dissolved in a solution

of DMSO/ethanol (1:1) (final concentration of 0.3

mg/ml). The glass substrate subjected to the treatment

25   with a silane coupling agent was soaked in this EMCS

solution for two hours to carry out the reaction

between the amino group of the silane coupling agent

covering the surface of the substrate and the

succinimide group in the EMCS solution. In association

with this reaction, the substrate is covered with EMCS

through the silane coupling agent. In the obtained

5   glass surface, a maleimide group derived from the EMCS

exists on the surface. The glass substrate taken out

after the reaction with the EMCS solution is cleaned

with distilled water, and is thereafter dried with

nitrogen gas. This glass substrate subjected to the

10   surface treatment for introducing a maleimide group

will be used for a binding reaction with DNA described

later.

　　　　3.   Synthesis of DNA for fixing glass substrates

　　　　Oligonucleotide having a base sequence of the

15   following Sequence 1 is chemically synthesized for

fixation on the glass substrate. This sequence 1 is a

18 mer sequence including in its central part a base

sequence with a base length of 6 to code 248th and

249th amino acids in an amino acid sequence of a gene

20   product (peptide chain) that is coded by the p 53 gene

known as a tumor suppressor gene. Also, A SH group is

introduced in its 5' end for fixation on the glass

substrate.

　　　　Sequence 1   5' HS-GATGGGCCTCCGGTTCAT3'

25   The SH group is introduced by using a commercially

available reagent Thiol-Modifier (manufactured by

GlenResearch Co., Ltd.) on a DNA automatic synthesizing

apparatus. Subsequently, normal deprotction was carried out to recover DNA, and the DNA was purified by high speed liquid chromatography, and was then used in the following processes.

5   4. Discharging of DNA using a BJ printer head and binding thereof to a substrate

The above described synthetic oligonucleotide (DNA) was dissolved in water, and the solution was diluted to the a concentration of 8 µM using SG Clear

10   (a solution containing 7.5% of glycerin, 7.5% of urea, 7.5% of thiodiglycol and 1% of acetylenol EH).

100 µl of this oligonucleotide solution was charged into the nozzle of BJ printer head BC 62 (manufactured by Canon. Inc.) with the nozzle modified

15   so that it is suitable for a small amount of samples (discharged amount). This modified printer head was set in a plotting apparatus to perform printing over the surface of the glass substrate as an area of "solid print" of 2 mm square with the oligonucleotide

20   solution. Furthermore, the modified printer head that was used is used for bubble jet type ink jet printing and enables printing to be performed at a resolution of 360 × 720 dpi.

Thereafter, the glass substrate coated with the

25   oligonucleotide solution was left in a humidifier chamber for 30 minutes to carry out a reaction between the maleimide group on the surface of the substrate and

the thiol group (HS-) of oligonucleotide. Thereafter, unreacted oligonucleotide was removed. The prepared substrate to detect is a substrate with the synthetic DNA (oligonucleotide) of the above described Sequence 1

5   bound to a predetermined section of 2 mm square on the glass substrate through covalent bond.

(Example 4)

Supply of cDNA solution to the surface of the substrate with oligonucleotide bound thereto and

10   hybridization reaction.

From various kinds of cDNA libraries obtained from tumor tissues, p 53 gene fragments were PCR-amplified, and then only one side chains were reamplified using primers marked in advance to prepare marked single-

15   strain cDNA for use as test samples. The hybridization reaction was carried out between this marked single strain DNA derived from the p 53 gene and the DNA probe bound on the detection substrate prepared in Example 3.

1. Preparation of test samples

20   From 64 types of cDNA libraries obtained from tumor tissues, p 53 gene fragments were obtained by the PCR reaction.

Specifically, first, all RNA samples were separated/collected from respective tissues collected

25   with the biopsy, using Catrimox-14 (Biotechnology Co., Ltd.). On the basis of the all RNA sample solutions, a c-DNA library was prepared using First-Strand cDNA

Synthesis Kit (manufactured by Life Science Co., Ltd.).

A primer for amplifying p 53 genes was added to this

cDNA library to amplify P 53 gene fragments. With this

PCR amplification product as a template, the marked

5   five side primer was used to carry out the PCR reaction

(DNA synthetic reaction) to amplify only one side

chains. By this amplification, marked single strain

DNA derived from the p 53 gene can be prepared.

     (1) Amplification of p 53 gene fragments having a

10   T3 binding site in the terminal by the PCR method.

     For using a primer for auto sequencers (Takara

Shuzo Co., Ltd) using T3 promoters as the above

described marked primer, a primer having a T3 site in

the terminal and having coupled to its downstream a

15   base sequence allowing the p 53 gene part to be

amplified was first synthesized. The PCR reaction was

carried out using this primer to obtain a PCR

amplification product having a T3 promoter site coupled

to the p 53 gene part.

20   In this example, for the five prime-end primer for

amplifying p 53 genes, the primer with a base sequence

having a T3 promoter site coupled to its five side (T3-

P53) was prepared. The base sequence is shown below.

     5'

25   AATTAACCCTCACTAAAGGGAACCTGAGGTTGGCTCTGACTGTACCACCATCC3'

     In the sequence, the underlined part on the side

of five prime-end represents a T3 polymerase binding

site. On the other hand, for a three prime-end primer
for amplification, a three prime-end primer attached in
a commercially available amplification kit, "Human p 53
Amplimer Set" of CLONTECH Co., Ltd. was used. For a
PCR reactive solution, "one shot LA PCR Mix" (Takara
Shuzo Co., Ltd.) was used.

The solution composition in the PCR reaction has:

one shot LA PCR Mix	25 µl
T3-P53 primer (20 µM)	1 µl
3' primer (20 µM)	1 µl
cDNA library solution	1 µl
DW	22 µl/50 µl,

and for the PCR cycle, a condition of conducting
cycles at 95° C for 30 seconds, at 55° C for 30 seconds
and at 72° C for 60 seconds at 29 times after thermal
denaturation at 95° C for 5 minutes and finally keeping
the solution at 72° C for five minutes was used, and the
reactant was stored at 4° C on a temporary basis after
it was cooled.

After the reaction, gel electrophoresis was
carried out to confirm a PCR product existing in the
region of molecular weight of about 300 mer. This PCR
product was purified with Micro Spin Column S200
(Pharmacia) to obtain p 53 gene fragments to which the
T3 primer can be coupled (T3-linked p 53 DNA).

(2) Synthesis of marked single strain DNA using
labeled T3 primers (Rho-T3).

With the p 53 gene fragment obtained in (1) as a matrix, single strain marked DNA was obtained with the PCR reaction , using a Rho-T3 primer (Takara Shuzo Co., Ltd.). The composition of the reactive solution had:

5

one shot LA PCR Mix	25 μl
Rho-T3primer (10 μM)	1 μl
T3-linked p 53 DNA	1 μl
DW	23 μl/50 μl,

and for the reaction cycle, a condition of

10 conducting cycles at 96° C for 30 seconds, at 50° C for 15 seconds and at 60° C for 4 minutes 24 times was used, and the reactant was stored at 4° C on a temporary basis after it was cooled. It was purified with Micro Spin Column S200, and thereafter gel electrophoresis was

15 carried out to confirm desired rhodamine labeled single strain DNA synthesized through the PCR reaction.

2.   Supply of test sample solution

Sodium chloride was added in the test sample obtained in the above described process, namely the

20 solution of rhodamine marked single strain DNA derived from the p 53 gene so that the final concentration of the solution was 1M. The solution of rhodamine marked single strain DNA derived from the p 53 gene, which had been prepared from 64 types of c DNA libraries was

25 injected into each well of a 96-hole microtiter plate. These solutions of rhodamine marked single strain DNA were spotted as an arrangement of 8 × 8 onto the

detection glass substrate with the DNA probe of

Sequence 1 obtained in Example 3 in the form of 2 mm

square, using a microarray preparing apparatus (pin

system) manufactured by Cartesian Technologies. The

5   diameter of each spot was 100 µm.

3. Hybridization reaction.

This detection substrate with total 64 types of

rhodamine marked single strain DNA solutions being

sample specimens spotted thereon was left in a

10   humidifier chamber set at 40° C to carry out a

hybridization reaction for 3 hours. Thereafter, the

detection substrate was washed with a 10 mM phosphate

buffer containing 100 mM NaCl to remove test samples

that had not been engaged in the formation of hybrid

15   substances.

After the hybridization reaction, the test sample

spotted in the form of a two-dimensional array of 8 × 8

was observed using an inverted fluorescence microscope

equipped with a filter set for excitation light and

20   fluorescence suitable for fluorescence marked

rhodamine. For the most part of the spots, red

fluorescence derived from fluorescence marked rhodamine

in association with the formation of hybrid substances

was observed. However, fluorescence intensity was weak

25   for six spots and no fluorescence was observed for one

spot.

For this, it can be considered that since in the p

53 gene derived from corresponding six types of tumor cells, variation occurs somewhere in the base sequence corresponding to the 248th and 249th of the amino acid sequence of the p 53 gene product (p 53 protein), the

5   amount of formed hybrid substances is small due to its mismatch, and in association therewith, the fluorescence intensity from the fluorescence mark is weak. For the test sample in which fluorescence was not observed, it can be considered from the fact that

10   hybrid substances were not formed that in p 53 cDNA fragments contained in the sample, deficiency occurs in the base sequence to code the above described 248th and 249th of the amino acid sequence, and consequently hybrid substances could not be formed.

15   (Example 5)

Preparation of array form spots of test samples on the probe matrix detection substrate with multiple oligonucleotides fixed thereon.

1.   Preparation of 64 probe matrices.

20   Processing was performed as in the case of Example 3 to prepare a glass substrate having a maleimide group. 64 DNA of which base sequences are shown in Table 2 were printed (applied) thereon in the area of 2 mm square, respectively, using a bubble jet printer

25   head similar to that of Example 3 to prepare a detection substrate on which sections with 64 types of prove DNAs fixed therein were arranged in a matrix

form.

Focusing attention on the 248th and 249th amino acids of the amino acid sequence of the gene product (p 53 protein) of the p 53 gene being a tumor suppressor gene, 64 DNAs of which base sequences are shown in Table 1 were selected on the basis of the base sequence to code these two amino acids so that a sequence with various kinds of base variations added thereto was obtained. Specifically, it is known that a case of frequent variation in the base sequence CGGAGG providing a base is the case where the first C of the CGG to code the 248th amino acid is changed to T, the second A is changed to G, and the third G of the AGG to code the 248th amino acid is changed to T. Thus, 64 probes were designed to provide sequences capable of being bound to base sequences with these bases at three positions varied in various kinds of forms.

Actually, it was a structure in which the total length of the probe was 18 mer, six bases including this variation were located in the center thereof, and common base sequences with base lengths of 6 were placed before and after the six bases. More specifically, the structure has a common sequence of ATGAAC from the side of the five prime-end, the base sequence of NNGAGN as a portion including the variation, and a common sequence of CCCATC on the side of three prime-end.

It was a base sequence a base sequence
complimentary to the sequence of
5'ATGAACNNGAGNCCCATC3'. That is, it was a probe
expressed by 5'GATGGGNCTCNNGTTCAT3'. Furthermore,

5   since it is a DNA probe, the portion denoted by N in
the above described base sequence refers to any one of
A, G, C and T that are four DNA nucleic acid bases.

Table 2

1	5'-GATGGGACTCAAGTTCAT-3'	33	5'-GATGGGCCTCAAGTTCAT-3'
2	5'-GATGGGACTCAGGTTCAT-3'	34	5'-GATGGGCCTCAGGTTCAT-3'
3	5'-GATGGGACTCACGTTCAT-3'	35	5'-GATGGGCCTCACGTTCAT-3'
4	5'-GATGGGACTCATGTTCAT-3'	36	5'-GATGGGCCTCATGTTCAT-3'
5	5'-GATGGGACTCGAGTTCAT-3'	37	5'-GATGGGCCTCGAGTTCAT-3'
6	5'-GATGGGACTCGGGTTCAT-3'	38	5'-GATGGGCCTCGGGTTCAT-3'
7	5'-GATGGGACTCGCGTTCAT-3'	39	5'-GATGGGCCTCGCGTTCAT-3'
8	5'-GATGGGACTCGTGTTCAT-3'	40	5'-GATGGGCCTCGTGTTCAT-3'
9	5'-GATGGGACTCCAGTTCAT-3'	41	5'-GATGGGCCTCCAGTTCAT-3'
10	5'-GATGGGACTCCGGTTCAT-3'	42	5'-GATGGGCCTCCGGTTCAT-3'
11	5'-GATGGGACTCCCGTTCAT-3'	43	5'-GATGGGCCTCCCGTTCAT-3'
12	5'-GATGGGACTCCTGTTCAT-3'	44	5'-GATGGGCCTCCTGTTCAT-3'
13	5'-GATGGGACTCTAGTTCAT-3'	45	5'-GATGGGCCTCTAGTTCAT-3'
14	5'-GATGGGACTCTGGTTCAT-3'	46	5'-GATGGGCCTCTGGTTCAT-3'
15	5'-GATGGGACTCTCGTTCAT-3'	47	5'-GATGGGCCTCTCGTTCAT-3'
16	5'-GATGGGACTCTTGTTCAT-3'	48	5'-GATGGGCCTCTTGTTCAT-3'
17	5'-GATGGGGCTCAAGTTCAT-3'	49	5'-GATGGGTCTCAAGTTCAT-3'
18	5'-GATGGGGCTCAGGTTCAT-3'	50	5'-GATGGGTTCTAGGTTCAT-3'
19	5'-GATGGGGCTCACGTTCAT-3'	51	5'-GATGGGTCTCACGTTCAT-3'
20	5'-GATGGGGCTCATGTTCAT-3'	52	5'-GATGGGTCTCATGTTCAT-3'
21	5'-GATGGGGCTCGAGTTCAT-3'	53	5'-GATGGGTCTCGAGTTCAT-3'
22	5'-GATGGGGCTCGGGTTCAT-3'	54	5'-GATGGGTCTCGGGTTCAT-3'
23	5'-GATGGGGCTCGCGTTCAT-3'	55	5'-GATGGGTCTCGCGTTCAT-3'
24	5'-GATGGGGCTCGTGTTCAT-3'	56	5'-GATGGGTCTCGTGTTCAT-3'
25	5'-GATGGGGCTCCAGTTCAT-3'	57	5'-GATGGGTCTCCAGTTCAT-3'
26	5'-GATGGGGCTCCGGTTCAT-3'	58	5'-GATGGGTCTCCGGTTCAT-3'
27	5'-GATGGGGCTCCCGTTCAT-3'	59	5'-GATGGGTCTCCCGTTCAT-3'
28	5'-GATGGGGCTCCTGTTCAT-3'	60	5'-GATGGGTCTCCTGTTCAT-3'
29	5'-GATGGGGCTCTAGTTCAT-3'	61	5'-GATGGGTCTCTAGTTCAT-3'
30	5'-GATGGGGCTCTGGTTCAT-3'	62	5'-GATGGGTCTCTGGTTCAT-3'
31	5'-GATGGGGCTCTCGTTCAT-3'	63	5'-GATGGGTCTCTCGTTCAT-3'
32	5'-GATGGGGCTCTTGTTCAT-3'	64	5'-GATGGGTCTCTTGTTCAT-3'

Then, for each of the 64 types of labeled prove

DNAs, a 8 µM solution containing glycerin, urea and

thiodiglycol in the final concentration of 7.5%,

respectively, and acetylenol EH in the final

5   concentration of 1% was prepared. As in the case of

Example 4, using BJ Printer Head BC 62 (manufactured by

Cannon Inc), different DNA probe solution was charged

by 100 µl in each of the six nozzles of the printer

head, and using a plurality of such printer heads, a

10 detection substrate with total 64 DNA probes applied to

and fixed in each section of 2 mm square in the form of

"solid print" and arranged in a matrix form (8 × 8) was

prepared. A schematic layout of the 64 DNA probes

arranged in a matrix form (8 × 8) on the detection

15 substrate is shown in FIG. 7.

2.   Preparation of array spots of test samples.

As in the case of Example 4, 64 types of labeled

cDNAs were spotted in the form of the two dimensional 8

× 8 array on each region of 2 mm square for fixing

20 probes. Specifically, as schematically shown in FIG.

8, a pin system array preparing apparatus was used to

form spots in the form of the two dimensional 8 × 8

array on the sections arranged in a matrix form (8 × 8)

in which each DNA probe was fixed.

25 3.   Hybridization reaction.

A hybridization reaction was carried out using

conditions and procedures similar to those of Example

4. The result thereof is shown in FIG. 9. In the
arrangement shown in FIG. 7, with respect to spots on
probes corresponding to the base sequence of the 42nd
normal gene, fluorescence intensity was weak for six
5   spots as in the case of Example 4. Also, no
fluorescence was observed for one spot. In addition
thereto, it was observed that fluorescent was emitted
from the spot at three points in the tenth probe
region, at two points in the 41st probe region, and at
10   one point in the 46th probe region, respectively.

Spot positions in which fluorescence in
association with the formation of hybrid substances was
observed in the prove region having these base
sequences including variations corresponded to spot
15   positions of weak fluorescence intensity in the probe
region having the above described 42nd original base
sequence. Thus, if the base sequences of the probes
are compared between both the regions, the base
sequence of the tenth probe is ACTCCG, the base
20   sequence of the 41st probe is the CCTCCA, and the base
sequence of the 46th probe is CCTCTG with respect to
the original base sequence of CCTCCG that the 42nd
probe has. For their complementary sequences, it can
be understood that with respect to the CGGAGG in the
25   42nd probe, the CGGAGT and G were changed to T in the
tenth probe, the TGGAGG and C were changed to T in the
41st probe, and the CAGAGG and G were changed to A in

the 46th probe. That is, it was confirmed that in test
samples forming hybrid substances with these tenth,
41st and 46th probes, cDNA fragments contained therein
derived from the p53 gene caused one base mismatch with
5   respect to the 42nd probe due to the above described
variations.

By this method, existence/not existence of
variations and types thereof could be detected at the
same time for all the 64 types of test samples.

10   (Example 6)

Preparation of a substrate for probe matrices
partitioned by patterns.

A glass substrate with an epoxy group introduced
to the surface and with black matrices for probe
15   matrices was prepared in accordance with the following
procedure.

1.   Introduction of an epoxy group to the surface
of the substrate.

A glass substrate made of synthetic quartz (50 mm
20   × 50 mm) was first subjected to ultrasonic cleaning
using a 2% sodium hydrate solution, and was then
subjected to UV ozone processing to clean the surface.
A 50% methanol solution containing 1% of silane
coupling agent (trade name: KBM 403; manufactured by
25   The Shin-Etsu Chemical Co., Ltd.) containing a silane
compound having an epoxy group bonded thereto (γ-
glycidoxypropyltrimethoxysilane) was stirred at room

temperature for three hours to perform preliminary treatment for hydrolyzing the methoxy group in the silane compound. This solution already subjected to the hydrolysis treatment was applied to the surface of the above described clean substrate with a spin coater, and was heated and dried at 100°C for 5 minutes to form a binding coating of the silane coupling agent on the surface of the substrate. Through the formation of this coating, the epoxy group contained in the silane compound was introduced to the surface of the substrate.

2. Formation of black matrices.

Then, A DEEP-UV resist containing carbon black (negative type resist for black matrices) (trade name: BK-739P; manufactured by Nippon Steel Chemical Co., Ltd.) was applied on the surface of the substrate with a spin coater so that the film thickness after curing was 5 μm, and it was heated for curing on a hotplate at 80°C for 5 minutes. By proximity exposure using a DEEP-UV aligner, a pattern was exposed to light using as an exposure mask a mask for negatives with patterning applied to a region of 10 mm × 10 mm so that the distance X between adjacent wells was 100 μm and the outer shape of the well was a square of 1 mm × 1 mm. Then, development was carried out with a developer of inorganic aqueous alkaline solution using a spin drier, and the substrate was washed with pure water to

remove the developer completely. Then, it was briefly

dried using the spin drier, and was thereafter heated

in a clean oven at 180°C for 30 minutes to fully cure

the resist. As a whole, a substrate with 400 wells

5   arranged in a predetermined arrangement and black

matrices (resist walls) partitioning adjacent wells was

obtained. Furthermore, the internal volume of each

well is calculated as 5 µl if the thickness of solution

is 5 µm. Also, in the surface of the prepared black

10   matrix, the angle of contact to water was 93 degrees

and wettability with water was significantly low, while

in the bottom of the well, the angle of contact to

water was 35 degrees and the wettability with water was

high.

15   3.   Fixation of probe DNA.

64 oligonucleotides of 18 mer with an amino group

bound to the hydroxyl group of the five prime-end

through a phosphate group and hexamethylene were

prepared as DNA probes. The 64 probes are same as

20   those prepared in Example 5 as to base sequences, but

are different in the sense that an amino group is

introduced in its five prime-end instead of a thiol

group.

5 µl of solution of these DNA probes was injected

25   into each well under a microscope, and was left in a

humidified chamber to allow the probe bind to the

substrate through the reaction between the amino group

of the five primer-end and the epoxy group on the substrate.

(Example 7)

Analysis of cDNA derived from the p 53 gene that has been prepared from mRNA, using the probe matrix substrate partitioned by the pattern that has been prepared in Example 6.

As in the case of Example 4, 64 types of labeled cDNAs were spotted in each probe region of 2 mm square as an arrangement of 8 × 8 spots as shown in FIG. 8, using a pin system array preparing apparatus.

A hybridization reaction was carried out by a method similar to that of example 4.

The obtained result was similar to that of Example 5.

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